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- (73) Patenthaver: **BASF Plant Science GmbH, , 67056 Ludwigshafen, Tyskland**
- (72) Opfinder: **CIRPUS, Petra, Landteilstr. 12, 68163 Mannheim, Tyskland**
BAUER, Jörg, Thorwaldsenstr. 4A, 67061 Ludwigshafen, Tyskland
- (74) Fuldmægtig i Danmark: **RWS Group, Europa House, Chiltern Park, Chiltern Hill, Chalfont St Peter, Bucks SL9 9FG, Storbritannien**
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Description

The present invention generally relates to the production of polyunsaturated fatty acids in transgenic organisms, by introducing, into the organism, the nucleic acids which code for polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, a $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase activity. These desaturases and elongases are advantageously derived from *Phytophthora sojae*.

The invention furthermore relates to the nucleic acid sequences, nucleic acid constructs, vectors and organisms comprising the nucleic acid sequences according to the invention, vectors comprising the nucleic acid sequences and/or the nucleic acid constructs and to transgenic plants comprise the abovementioned nucleic acid sequences, nucleic acid constructs and/or vectors.

A further part of the invention relates to fatty acid compositions produced by the process described here and to their use.

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and in the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Polyunsaturated fatty acids such as linoleic acid and linolenic acid are essential for mammals, since they cannot be produced by the latter. Polyunsaturated $\omega 3$ -fatty acids and $\omega 6$ -fatty acids are therefore an important constituent in animal and human nutrition.

Polyunsaturated long-chain $\omega 3$ -fatty acids such as eicosapentaenoic acid (= EPA, C20:5 $\Delta 5,8,11,14,17$) or docosahexaenoic acid (= DHA, C22:6 $\Delta 4,7,10,13,16,19$) are important components in human nutrition owing to their various roles in

health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A Lipids 30:1-14, 1995; Horrocks, LA and Yeo YK Pharmacol Res 40:211-225, 1999). This is why there is a demand for the production of polyunsaturated long-chain fatty acids.

Owing to the present-day composition of human food, an addition of polyunsaturated ω 3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, C22:6 ^{Δ 4,7,10,13,16,19}) or eicosapentaenoic acid (= EPA, C20:5 ^{Δ 5,8,11,14,17}) are added to infant formula to improve the nutritional value. The unsaturated fatty acid DHA is said to have a positive effect on the development and maintenance of brain functions.

Hereinbelow, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (poly unsaturated fatty acids, **PUFA**, long chain poly unsaturated fatty acids, **LCPUFA**).

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella and Schizochytrium or from oil-producing plants such as soybean, oilseed rape, algae such as Cryptocodinium or Phaeodactylum and others, where they are obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, such as, for example, fish. The free fatty acids are advantageously prepared by hydrolysis. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (= ARA, C20:4 ^{Δ 5,8,11,14}), dihomog γ -linolenic acid (C20:3 ^{Δ 8,11,14}) or docosapentaenoic acid (DPA, C22:5 ^{Δ 7,10,13,16,19}) are not synthesized in oil crop plants such as oilseed rape, soybean, sunflower or safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Depending on the intended use, oils with saturated or unsaturated fatty acids are preferred. In human nutrition, for example, lipids with unsaturated fatty acids, specifically
5 polyunsaturated fatty acids, are preferred. The polyunsaturated ω 3-fatty acids are said to have a positive effect on the cholesterol level in the blood and thus on the possibility of preventing heart disease. The risk of heart disease, stroke or hypertension can be reduced markedly by
10 adding these ω 3-fatty acids to the food. Also, ω 3-fatty acids have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis. They are therefore added to foodstuffs, specifically to dietetic
15 foodstuffs, or are employed in medicaments. ω 6-Fatty acids such as arachidonic acid tend to have a negative effect on these disorders in connection with these rheumatic diseases on account of our usual dietary intake.

20 ω 3- and ω 6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomog- γ -linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and
25 eicosapentaenoic acid. Eicosanoids (known as the PG₂ series) which are formed from ω 6-fatty acids generally promote inflammatory reactions, while eicosanoids (known as the PG₃ series) from ω 3-fatty acids have little or no proinflammatory effect.

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Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils
35 in various organisms with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describes a Δ 9-desaturase. WO 93/11245 claims a Δ 15-desaturase and WO 94/11516 a Δ 12-desaturase. Further desaturases are

described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 649-659. However, the biochemical characterization of the various desaturases has been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777-792). As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. $\Delta 6$ -Desaturases are described in WO 93/06712, US 5,614,393, US 5614393, WO 96/21022, WO 00/21557 and WO 99/27111 and the application for the production in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. In this context, the expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/64616 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ -linolenic acid and stearidonic acid. Moreover, a mixture of $\omega 3$ - and $\omega 6$ -fatty acids was obtained, as a rule.

A large number of attempts have been made in the past of obtaining elongase genes. Millar and Kunst, 1997 (Plant Journal 12:121-131) and Millar et al. 1999, (Plant Cell 11:825-838) describe the characterization of elongases from plants for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the production of wax in plants (C₂₈-C₃₂), respectively. Descriptions on the synthesis of arachidonic acid and EPA are found, for example, in WO0159128, WO0012720,

W002077213 and W00208401. The synthesis of polyunsaturated C24-fatty acids is described for example in Tvrdik et al 2000, JCB 149:707-717 or W00244320.

5 Especially suitable microorganisms for the production of PUFAs are microalgae such as *Phaeodactylum tricornutum*, *Porphyridium* species, *Thraustochytrium* species, *Schizochytrium* species or *Cryptothecodinium* species, ciliates such as *Stylonychia* or *Colpidium*, fungi such as *Mortierella*, *Entomophthora* or *Mucor*
10 and/or mosses such as *Physcomitrella*, *Ceratodon* and *Marchantia* (R. Vazhappilly & F. Chen (1998) *Botanica Marina* 41: 553-558; K. Totani & K. Oba (1987) *Lipids* 22: 1060-1062; M. Akimoto et al. (1998) *Appl. Biochemistry and Biotechnology* 73: 269-278). Strain selection has resulted in the development of a number
15 of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult
20 process. This is why recombinant methods as described above are preferred whenever possible. However, only limited amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms, and, depending on the microorganism used,
25 these are generally obtained as fatty acid mixtures of, for example, EPA, DPA and ARA.

A variety of synthetic pathways is being discussed for the synthesis of arachidonic acid, eicosapentaenoic acid (EPA) and
30 docosahexaenoic acid (DHA) (figure 1). Thus, EPA or DHA are produced in marine bacteria such as *Vibrio* sp. or *Shewanella* sp. via the polyketide pathway (Yu, R. et al. *Lipids* 35:1061-1064, 2000; Takeyama, H. et al. *Microbiology* 143:2725-2731, 1997).

35

An alternative strategy is the alternating activity of desaturases and elongases (Zank, T.K. et al. *Plant Journal* 31:255-268, 2002; Sakuradani, E. et al. *Gene* 238:445-453,

1999). A modification of the above-described pathway by $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and $\Delta 4$ -desaturase is the Sprecher synthetic pathway (Sprecher 2000, Biochim. Biophys. Acta 1486:219-231) in mammals. Instead of
5 the $\Delta 4$ -desaturation, a further elongation step is effected here to give C_{24} , followed by a further $\Delta 6$ -desaturation and finally β -oxidation to give the C_{22} chain length. Thus what is known as Sprecher synthetic pathway (see figure 1) is, however, not suitable for the production in plants and
10 microorganisms since the regulatory mechanisms are not known.

As can be seen from figure 1, the production of longer-chain polyunsaturated fatty acids such as arachidonic acid, EPA or in particular DHA ($C_{22:6}$ n-3) require, besides the
15 desaturases, also elongases, which elongate unsaturated fatty acids with double bonds, for example in delta-9, delta-6 or delta-5 position by at least two carbon atoms. One distinguishes between two different types of elongase, depending on their function. The type I-elongases, which are
20 widespread in the animal kingdom, only have poor substrate specificity, that is to say they elongate a series of different unsaturated fatty acids. Type II-elongases are distinguished by a much higher substrate specificity. They convert only few fatty acids with double bonds in specific
25 positions.

WO 2005/012316 describes some of the abovementioned desaturases and elongases. Specifically, WO 2005/012316 discloses first type II-elongases which specifically elongate
30 fatty acids with a double bond in the delta-5 position.

Depending on their desaturation pattern, the polyunsaturated fatty acids can be divided into two large classes, viz. $\omega 6$ - or $\omega 3$ -fatty acids, which differ with regard to their metabolic
35 and functional activities (fig. 1).

The starting material for the $\omega 6$ -metabolic pathway is the fatty acid linoleic acid ($18:2^{\Delta 9,12}$) while the $\omega 3$ -pathway

proceeds via linolenic acid (18:3^{Δ^{9,12,15}}). Linolenic acid is formed by the activity of an ω3-desaturase (Tocher et al. 1998, Prog. Lipid Res. 37, 73-117 ; Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113).

5

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω3-desaturase) and must take up these fatty acids (essential fatty acids) via the food. Starting with these precursors, the physiologically important polyunsaturated fatty acids arachidonic acid (= ARA, 20:4^{Δ^{5,8,11,14}}), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, 20:5^{Δ^{5,8,11,14,17}}) and docosahexaenoic acid (DHA, 22:6^{Δ^{4,7,10,13,17,19}}) are then synthesized via the sequence of desaturase and elongase reactions. The application of ω3-fatty acids shows the therapeutic activity described above in the treatment of cardiovascular diseases (Shimikawa 2001, World Rev. Nutr. Diet. 88, 100-108), inflammations (Calder 2002, Proc. Nutr. Soc. 61, 345-358) and arthritis (Cleland and James 2000, J. Rheumatol. 27, 2305-2307).

Higher plants comprise polyunsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). ARA, EPA and DHA are found not at all in the seed oil of higher plants, or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales [New Dictionary of Vegetable Oils]. Technique & Documentation - Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflower and soybeans, would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically in this way. To this end, it is advantageous to introduce, into oil crops, genes which code for enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. WO 2005/012316 describes such an approach. However, the disadvantage of the path described in WO 2005/012316 is that the yield of the desired fatty acids is

still too low to be exploited industrially. In addition, not only the desired fatty acids such as ARA, EPA or DHA are generated, but undesired secondary reactions also give rise to fatty acids which further reduce the yield and contaminate the product formed.

An advantageous process for the production of polyunsaturated fatty acids should therefore combine in itself as many as possible of the following properties:

10

- high specificity of the desaturases and elongases used for the production of polyunsaturated fatty acids,

15

- high synthesis rate of the desaturases and elongases used,

- synthesis of, if possible, only one polyunsaturated fatty acids such as ARA, EPA or DHA,

20

- high yield of polyunsaturated fatty acids such as ARA, EPA or DHA or their mixtures,

- the lowest possible amount of undesired secondary products, if any,

25

- no production of unnatural fatty acids, i.e. fatty acids which do not occur naturally,

- synthesis of the polyunsaturated fatty acids advantageously only in the triglycerides.

30

In order to make possible a fortification of the food and of the feed with these polyunsaturated fatty acids, there is therefore a great demand for a simple, inexpensive process for the production of these polyunsaturated fatty acids, specifically in eukaryotic systems.

It was therefore an object to develop a simple, inexpensive process which has as many as possible of the abovementioned

advantageous properties. Another object of the invention was to specify nucleic acids, proteins, gene constructs, vectors and transgenic organisms, preferably plants and their parts, which are useful in the context of the production of polyunsaturated fatty acids.

The object is achieved by the subject matter of the patent claims.

Also provided is a process for the production of polyunsaturated C₂₀- or C₂₂-fatty acids with at least four double bonds in transgenic plants with a content of at least 15% by weight based on the total triglyceride content of the transgenic plants, which comprises the following process steps:

a) introducing, into the transgenic plant, a nucleic acid construct which comprises nucleic acid sequences which codes for a $\Delta 6$ -desaturase, a $\Delta 6$ -elongase and a $\Delta 5$ -desaturase, or

b) introducing, into the transgenic plant, a nucleic acid construct which comprises nucleic acid sequences which codes for a $\Delta 6$ -desaturase, a $\Delta 6$ -elongase, a $\Delta 5$ -desaturase, a $\Delta 12$ -desaturase and $\omega 3$ -desaturase, or

c) introducing, into the transgenic plant, a nucleic acid construct which comprises nucleic acid sequences which codes for a $\Delta 6$ -desaturase, a $\Delta 6$ -elongase, a $\Delta 5$ -desaturase, a $\Delta 5$ -elongase and $\Delta 4$ -desaturase, or

d) introducing, into the transgenic plant, a nucleic acid construct which comprises nucleic acid sequences which codes for a $\Delta 6$ -desaturase, a $\Delta 6$ -elongase, a $\Delta 5$ -desaturase, a $\Delta 5$ -elongase, $\Delta 4$ -desaturase, a $\Delta 12$ -desaturase and $\omega 3$ -desaturase, and

e) obtaining the oils or lipids from the plants.

The C₂₀- and C₂₂-polyunsaturated fatty acids produced in the process advantageously comprise at least four, five or six double bonds. The fatty acids especially advantageously comprise five or six double bonds. Saturated fatty acids are advantageously converted to a minor degree, or not at all, by the nucleic acids used in the process. To a minor degree is to be understood as meaning that the saturated fatty acids are reacted with less than 5% of the activity, advantageously less than 3%, especially advantageously with less than 2%, very especially preferably with less than 1, 0.5, 0.25 or 0.125% of the activity in comparison with polyunsaturated fatty acids. These fatty acids which have been produced can be produced in the process as a single product or be present in a fatty acid mixture.

The nucleic acid sequences used in the process are isolated nucleic acid sequences which code for polypeptides with Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase, Δ 12-desaturase and/or ω 3-desaturase activity.

Nucleic acid sequences which are advantageously used in the process are those which code for polypeptides with Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase, Δ 12-desaturase and/or ω 3-desaturase activity, selected from the group consisting of:

SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25.

The polyunsaturated C₂₀- or C₂₂-fatty acids with at least four double bonds which are produced in the process are especially advantageously the fatty acids arachidonic acid (= ARA), eicosapentaenoic acid (= EPA) or docosahexaenoic acid (= DHA). In an advantageous embodiment of the process, the polyunsaturated C₂₀- or C₂₂-fatty acids with at least four double bonds is arachidonic acid. In another advantageous

embodiment, the polyunsaturated C₂₀- or C₂₂-fatty acids with at least four double bonds which are produced are eicosapentaenoic acid or docosahexaenoic acid or their mixtures. Mixtures of ARA, EPA and DHA can also be produced by
5 the process.

Advantageously, arachidonic acid or eicosapentaenoic acid with a content of at least 15, 16, 17, 18, 19 or 20% by weight, preferably 25, 30, 35 or 40% by weight, especially preferably
10 45, 50, 55 or 60% by weight, based on the total triglyceride content, are produced in the transgenic plant in the process.

In another advantageous embodiment of the process, docosahexaenoic acid with a content of at least 4, 5 or 6% by weight, advantageously with a content of 7, 8, 9 or 10% by
15 weight, based on the total triglyceride content, is produced in the transgenic plant.

It is especially advantageous that, in the process, less than
20 0.5, 0.4, 0.3, 0.2 or 0.1% by weight, advantageously less than 0.09, 0.08, 0.07, 0.06 or 0.05% by weight, especially advantageously less than 0.04, 0.03, 0.02 or 0.01% by weight, based on the total fatty acid content of the triglycerides, of a polyunsaturated fatty acid selected from the group
25 consisting of the C₂₂:4^{Δ^{7,10,13,16}}-, C₂₂:5^{Δ^{4,7,10,13,16}}- or C₂₂:5^{Δ^{7,10,13,16,19}}-fatty acid should be present in the triglycerides.

The polyunsaturated fatty acids produced in the process are
30 advantageously bound in triacylglycerides, but may also occur in the organisms as free fatty acids or else bound in the form of other fatty acid esters. In this context, they may be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different
35 glycerides.

The process advantageously yields fatty acid esters with polyunsaturated C₂₀- and/or C₂₂-fatty acid molecules with at

least four double bonds in the fatty acid ester, advantageously with at least five or six double bonds in the fatty acid ester. This leads to the synthesis of ω 3-eicosatetraenoic acid (= ETA, C20:4 ^{Δ 5,8,11,14}), arachidonic acid (ARA, C20:4 ^{Δ 5,8,11,14}), eicosapentaenoic acid (EPA, C20:5 ^{Δ 5,8,11,14,17}), ω 6-docosapentaenoic acid (C22:5 ^{Δ 4,7,10,13,16}), ω 6-docosatetraenoic acid (C22:4 ^{Δ 7,10,13,16}), ω 3-docosapentaenoic acid (= DPA, C22:5 ^{Δ 7,10,13,16,19}), docosahexaenoic acid (= DHA, C22:6 ^{Δ 4,7,10,13,16,19}) or mixtures of these ARA, EPA and/or DHA are preferably produced. In an especially preferred embodiment of the process, ω 6-fatty acids, advantageously arachidonic acid, are produced. In another especially preferred embodiment of the process, ω 3-fatty acids such as EPA and/or DHA are produced.

The process, as described above, especially advantageously yields polyunsaturated fatty acids selected from the group consisting of ω 6-docosatetraenoic acid C22:4 ^{Δ 7,10,13,16}, ω 6-docosapentaenoic acid C22:5 ^{Δ 4,7,10,13,16} or ω 3-docosapentaenoic acid C22:5 ^{Δ 7,10,13,16,19} in the triglycerides with less than 0.5% by weight based on the total fatty acid content of the triglycerides.

The fatty acid esters with polyunsaturated C₂₀- and/or C₂₂-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least four, five or six, preferably five or six double bonds, from the organisms which have been used for the preparation of the fatty acid esters; advantageously, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine,

especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the organisms, advantageously the plants as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

The process yields the LCPUFAs, such as arachidonic acid and eicosapentaenoic acid produced in a content of at least 15% by weight, advantageously at least 16 or 17% by weight, preferably at least 18, 19 or 20% by weight, especially preferably at least 21, 22, 23, 24 or 25% by weight, most preferably at least 26, 27, 28, 29 or 30% by weight based on the total fatty acids in the transgenic organisms, advantageously in a transgenic plant. In this context, it is advantageous to convert C₁₈- and/or C₂₀-fatty acids which are present in the host organisms to at least 10%, preferably to at least 20%, especially preferably to at least 30%, most preferably to at least 40% to give the corresponding products such as ARA, EPA and/or DHA. The fatty acids are advantageously produced in bound form. These unsaturated fatty acids can, with the aid of the nucleic acids used in the process, be positioned at the sn1, sn2 and/or sn3 position of the advantageously produced triglycerides. Since a plurality of reaction steps are performed by the starting compounds linoleic acid (C₁₈:2) and linolenic acid (C₁₈:3) in the process, the end products of the process such as, for example, arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosapentaenoic acid (DHA) are not obtained as absolutely pure products; minor traces of the precursors are always present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting organism and the starting plant, the end products such as ARA, EPA or

DHA are generally present as mixtures. The precursors linoleic acid and/or linolenic acid should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, especially preferably not to more than 10% by weight, most preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only ARA, ARA and EPA, EPA and DHA or only DHA, bound or as free acids, are produced as end products in a transgenic plant owing to the process. If the compounds ARA, EPA and DHA are produced simultaneously, they are advantageously produced in a ratio of at least 1:1:2 (EPA:ARA:DHA), advantageously of at least 1:1:3, preferably 1:1:4, especially preferably 1:1:5. If ARA and EPA are produced in the process, they are advantageously produced in a ratio of at least 5:1 to at least 1:5. If EPA and DHA are produced in the process, they are advantageously produced in a ratio of at least 5:1 to at least 1:5.

Fatty acid esters or fatty acid mixtures produced by the process advantageously comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Advantageous polyunsaturated fatty acids which are present in the fatty acid esters or fatty acid mixtures are preferably at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% of arachidonic acid, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process advantageously comprise fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterulic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenododecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid),

6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13c-octadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), puniic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The abovementioned fatty acids are, as a rule, advantageously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process, that is to say that, based on the total fatty acids, they occur to less than 30%, preferably to less than 25%, 24%, 23%, 22% or 21%, especially preferably to less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably to less than 4%, 3%, 2% or 1%. The fatty acid esters or fatty acid mixtures produced by the process advantageously comprise less than 0.1%, based on the total fatty acids, or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, C22:5^{A4,8,12,15,21}) and no nisinic acid (tetracosahexaenoic acid, C23:6^{A3,8,12,15,18,21}).

Owing to the nucleic acid sequences according to the invention, or the nucleic acid sequences used in the process, an increase in the yield of polyunsaturated fatty acids of at least 50%, advantageously of at least 80%, especially advantageously of at least 100%, very especially advantageously of at least 150%, in comparison with the nontransgenic plant such as Arabidopsis, linseed, oilseed rape, soybean or Camelina can be obtained when compared by GC analysis.

Chemically pure polyunsaturated fatty acids or fatty acid compositions can also be synthesized by the processes described above. To this end, the fatty acids or the fatty acid compositions are isolated from the plant, in a known manner, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

Suitable organisms for the production in the process are, in principle, all plants. Oil crop plants or useful plants are advantageously used in the process.

Plants which are suitable are, in principle, all those plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Advantageous plants are selected from the group of the plant families Adelotheceaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Crypthecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes.

Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheceaceae such as the genera *Physcomitrella*, for example the genus and species *Physcomitrella patens*, Anacardiaceae such as the genera *Pistacia*, *Mangifera*, *Anacardium*, for example the genus and species *Pistacia vera* [pistachio], *Mangifera indica* [mango] or *Anacardium occidentale* [cashew], Asteraceae, such as the genera *Calendula*, *Carthamus*, *Centaurea*, *Cichorium*, *Cynara*, *Helianthus*, *Lactuca*, *Locusta*, *Tagetes*, *Valeriana*, for example the genus and species *Calendula officinalis* [common marigold],

Carthamus tinctorius [safflower], Centaurea cyanus
 [cornflower], Cichorium intybus [chicory], Cynara scolymus
 [artichoke], Helianthus annuus [sunflower], Lactuca sativa,
 Lactuca crispera, Lactuca esculenta, Lactuca scariola L. ssp.
 5 sativa, Lactuca scariola L. var. integrata, Lactuca scariola
 L. var. integrifolia, Lactuca sativa subsp. romana, Locusta
 communis, Valeriana locusta [salad vegetables], Tagetes
 lucida, Tagetes erecta or Tagetes tenuifolia [african or
 french marigold], Apiaceae, such as the genus Daucus, for
 10 example the genus and species Daucus carota [carrot],
 Betulaceae, such as the genus Corylus, for example the genera
 and species Corylus avellana or Corylus colurna [hazelnut],
 Boraginaceae, such as the genus Borago, for example the genus
 and species Borago officinalis [borage], Brassicaceae, such as
 15 the genera Brassica, Camelina, Melanosinapis, Sinapis,
 Arabidopsis, for example the genera and species Brassica
 napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis
 Brassica juncea, Brassica juncea var. juncea, Brassica juncea
 var. crispifolia, Brassica juncea var. foliosa, Brassica
 20 nigra, Brassica sinapioides, Camelina sativa, Melanosinapis
 communis [mustard], Brassica oleracea [fodder beet] or
 Arabidopsis thaliana, Bromeliaceae, such as the genera Anana,
 Bromelia (pineapple), for example the genera and species Anana
 comosus, Ananas ananas or Bromelia comosa [pineapple],
 25 Caricaceae, such as the genus Carica, such as the genus and
 species Carica papaya [pawpaw], Cannabaceae, such as the genus
 Cannabis, such as the genus and species Cannabis sativa
 [hemp], Convolvulaceae, such as the genera Ipomea,
 Convolvulus, for example the genera and species Ipomoea
 30 batatas, Ipomoea pandurata, Convolvulus batatas, Convolvulus
 tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea
 triloba or Convolvulus panduratus [sweet potato, batate],
 Chenopodiaceae, such as the genus Beta, such as the genera and
 species Beta vulgaris, Beta vulgaris var. altissima, Beta
 35 vulgaris var. Vulgaris, Beta maritima, Beta vulgaris var.
 perennis, Beta vulgaris var. conditiva or Beta vulgaris var.
 esculenta [sugarbeet], Cryptocodiniaceae, such as the genus
 Cryptocodium, for example the genus and species

Cryptecodinium cohnii, Cucurbitaceae, such as the genus
Cucurbita, for example the genera and species *Cucurbita maxima*,
Cucurbita mixta, *Cucurbita pepo* or *Cucurbita moschata*
 [pumpkin/squash], Cymbellaceae, such as the genera *Amphora*,
 5 *Cymbella*, *Okedenia*, *Phaeodactylum*, *Reimeria*, for example the
 genus and species *Phaeodactylum tricornutum*, Ditrichaceae,
 such as the genera *Ditrichaceae*, *Astomiopsis*, *Ceratodon*,
Chrysoblastella, *Ditrichum*, *Distichium*, *Eccremidium*,
Lophidion, *Philibertiella*, *Pleuridium*, *Saelania*, *Trichodon*,
 10 *Skottsbergia*, for example the genera and species *Ceratodon*
antarcticus, *Ceratodon columbiae*, *Ceratodon heterophyllus*,
Ceratodon purpurascens, *Ceratodon purpureus*, *Ceratodon*
purpureus ssp. *convolutus*, *Ceratodon purpureus* ssp.
stenocarpus, *Ceratodon purpureus* var. *rotundifolius*, *Ceratodon*
 15 *ratodon*, *Ceratodon stenocarpus*, *Chrysoblastella chilensis*,
Ditrichum ambiguum, *Ditrichum brevisetum*, *Ditrichum*
crispatissimum, *Ditrichum difficile*, *Ditrichum falcifolium*,
Ditrichum flexicaule, *Ditrichum giganteum*, *Ditrichum*
heteromallum, *Ditrichum lineare*, *Ditrichum lineare*, *Ditrichum*
 20 *montanum*, *Ditrichum montanum*, *Ditrichum pallidum*, *Ditrichum*
punctulatum, *Ditrichum pusillum*, *Ditrichum pusillum* var.
tortile, *Ditrichum rhynchostegium*, *Ditrichum schimperii*,
Ditrichum tortile, *Distichium capillaceum*, *Distichium hagenii*,
Distichium inclinatum, *Distichium macounii*, *Eccremidium*
 25 *floridanum*, *Eccremidium whiteleggei*, *Lophidion strictus*,
Pleuridium acuminatum, *Pleuridium alternifolium*, *Pleuridium*
holdridgei, *Pleuridium mexicanum*, *Pleuridium ravenelii*,
Pleuridium subulatum, *Saelania glaucescens*, *Trichodon*
borealis, *Trichodon cylindricus* or *Trichodon cylindricus* var.
 30 *oblongus*, Elaeagnaceae, such as the genus *Elaeagnus*, for
 example the genus and species *Olea europaea* [olive],
 Ericaceae, such as the genus *Kalmia*, for example the genera
 and species *Kalmia latifolia*, *Kalmia angustifolia*, *Kalmia*
microphylla, *Kalmia polifolia*, *Kalmia occidentalis*, *Cistus*
 35 *chamaerhodendros* or *Kalmia lucida* [mountain laurel],
 Euphorbiaceae, such as the genera *Manihot*, *Janipha*, *Jatropha*,
Ricinus, for example the genera and species *Manihot*
utilissima, *Janipha manihot*, *Jatropha manihot*, *Manihot aipil*,

Manihot dulcis, *Manihot manihot*, *Manihot melanobasis*, *Manihot*
esculenta [cassava] or *Ricinus communis* [castor-oil plant],
 Fabaceae, such as the genera *Pisum*, *Albizia*, *Cathormion*,
Feuillea, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicajo*,
 5 *Glycine*, *Dolichos*, *Phaseolus*, soybean, for example the genera
 and species *Pisum sativum*, *Pisum arvense*, *Pisum humile* [pea],
Albizia berteriana, *Albizia julibrissin*, *Albizia lebbeck*,
Acacia berteriana, *Acacia littoralis*, *Albizia berteriana*,
Albizia berteriana, *Cathormion berteriana*, *Feuillea*
 10 *berteriana*, *Inga fragrans*, *Pithecellobium berterianum*,
Pithecellobium fragrans, *Pithecolobium berterianum*,
Pseudalbizia berteriana, *Acacia julibrissin*, *Acacia nemu*,
Albizia nemu, *Feuillea julibrissin*, *Mimosa julibrissin*,
Mimosa speciosa, *Sericanrda julibrissin*, *Acacia lebbeck*,
 15 *Acacia macrophylla*, *Albizia lebbeck*, *Feuillea lebbeck*, *Mimosa*
lebbeck, *Mimosa speciosa* [siris tree], *Medicago sativa*,
Medicago falcata, *Medicago varia* [alfalfa] *Glycine max*
Dolichos soja, *Glycine gracilis*, *Glycine hispida*, *Phaseolus*
max, *Soja hispida* or *Soja max* [soybean], Funariaceae, such as
 20 the genera *Aphanorrhagma*, *Entosthodon*, *Funaria*,
Physcomitrella, *Physcomitrium*, for example the genera and
 species *Aphanorrhagma serratum*, *Entosthodon attenuatus*,
Entosthodon bolanderi, *Entosthodon bonplandii*, *Entosthodon*
californicus, *Entosthodon drummondii*, *Entosthodon jamesonii*,
 25 *Entosthodon leibergii*, *Entosthodon neoscoticus*, *Entosthodon*
rubrisetus, *Entosthodon spathulifolius*, *Entosthodon tucsoni*,
Funaria americana, *Funaria bolanderi*, *Funaria calcarea*,
Funaria californica, *Funaria calvescens*, *Funaria convoluta*,
Funaria flavicans, *Funaria groutiana*, *Funaria hygrometrica*,
 30 *Funaria hygrometrica* var. *arctica*, *Funaria hygrometrica* var.
calvescens, *Funaria hygrometrica* var. *convoluta*, *Funaria*
hygrometrica var. *muralis*, *Funaria hygrometrica* var.
utahensis, *Funaria microstoma*, *Funaria microstoma* var.
obtusifolia, *Funaria muhlenbergii*, *Funaria orcuttii*, *Funaria*
 35 *plano-convexa*, *Funaria polaris*, *Funaria ravenelii*, *Funaria*
rubriseta, *Funaria serrata*, *Funaria sonora*, *Funaria*
sublimbatus, *Funaria tucsoni*, *Physcomitrella californica*,
Physcomitrella patens, *Physcomitrella readeri*, *Physcomitrium*

australe, *Physcomitrium californicum*, *Physcomitrium*
collenchymatum, *Physcomitrium coloradense*, *Physcomitrium*
cupuliferum, *Physcomitrium drummondii*, *Physcomitrium*
eurystomum, *Physcomitrium flexifolium*, *Physcomitrium hookeri*,
 5 *Physcomitrium hookeri* var. *serratum*, *Physcomitrium immersum*,
Physcomitrium kellermanii, *Physcomitrium megalocarpum*,
Physcomitrium pyriforme, *Physcomitrium pyriforme* var.
serratum, *Physcomitrium rufipes*, *Physcomitrium sandbergii*,
Physcomitrium subsphaericum, *Physcomitrium washingtoniense*,
 10 Geraniaceae, such as the genera *Pelargonium*, *Cocos*, *Oleum*, for
 example the genera and species *Cocos nucifera*, *Pelargonium*
grossularioides or *Oleum cocois* [coconut], Gramineae, such as
 the genus *Saccharum*, for example the genus and species
Saccharum officinarum, Juglandaceae, such as the genera
 15 *Juglans*, *Wallia*, for example the genera and species *Juglans*
regia, *Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans*
cinerea, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*,
Juglans hindsii, *Juglans intermedia*, *Juglans jamaicensis*,
Juglans major, *Juglans microcarpa*, *Juglans nigra* or *Wallia*
 20 *nigra* [walnut], Lauraceae, such as the genera *Persea*, *Laurus*,
 for example the genera and species *Laurus nobilis* [bay],
Persea americana, *Persea gratissima* or *Persea persea*
 [avocado], Leguminosae, such as the genus *Arachis*, for example
 the genus and species *Arachis hypogaea* [peanut], Linaceae,
 25 such as the genera *Adenolinum*, for example the genera and
 species *Linum usitatissimum*, *Linum humile*, *Linum austriacum*,
Linum bienne, *Linum angustifolium*, *Linum catharticum*, *Linum*
flavum, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum*
lewisii, *Linum narbonense*, *Linum perenne*, *Linum perenne* var.
 30 *lewisii*, *Linum pratense* or *Linum trigynum* [linseed],
 Lythrarieae, such as the genus *Punica*, for example the genus
 and species *Punica granatum* [pomegranate], Malvaceae, such as
 the genus *Gossypium*, for example the genera and species
Gossypium hirsutum, *Gossypium arboreum*, *Gossypium barbadense*,
 35 *Gossypium herbaceum* or *Gossypium thurberi* [cotton],
 Marchantiaceae, such as the genus *Marchantia*, for example the
 genera and species *Marchantia berteroana*, *Marchantia foliacea*,
Marchantia macropora, Musaceae, such as the genus *Musa*, for

example the genera and species *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa* spp. [banana], Onagraceae, such as the genera *Camissonia*, *Oenothera*, for example the genera and species *Oenothera biennis* or *Camissonia brevipes* [evening primrose], Palmae, such as the genus *Elacis*, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as the genus *Papaver*, for example the genera and species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* [poppy], Pedaliaceae, such as the genus *Sesamum*, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, for example the genera and species *Piper aduncum*, *Piper amalago*, *Piper angustifolium*, *Piper auritum*, *Piper betel*, *Piper cubeba*, *Piper longum*, *Piper nigrum*, *Piper retrofractum*, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata* [cayenne pepper], Poaceae, such as the genera *Hordeum*, *Secale*, *Avena*, *Sorghum*, *Andropogon*, *Holcus*, *Panicum*, *Oryza*, *Zea* (maize), *Triticum*, for example the genera and species *Hordeum vulgare*, *Hordeum jubatum*, *Hordeum murinum*, *Hordeum secalinum*, *Hordeum distichon*, *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, *Hordeum secalinum* [barley], *Secale cereale* [rye], *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. *sativa*, *Avena hybrida* [oats], *Sorghum bicolor*, *Sorghum halepense*, *Sorghum saccharatum*, *Sorghum vulgare*, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*, *Sorghum miliaceum*, *Panicum militaceum* [millet], *Oryza sativa*, *Oryza latifolia* [rice], *Zea mays* [maize] *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* [wheat], *Porphyridiaceae*, such as the genera *Chroothece*, *Flintiella*, *Petrovanella*, *Porphyridium*, *Rhodella*, *Rhodosorus*, *Vanhoeffenia*, for example the genus and species *Porphyridium*

cruentum, Proteaceae, such as the genus *Macadamia*, for example
 the genus and species *Macadamia intergrifolia* [macadamia],
 Prasinophyceae, such as the genera *Nephroselmis*,
Prasinococcus, *Scherffelia*, *Tetraselmis*, *Mantoniella*,
 5 *Ostreococcus*, for example the genera and species *Nephroselmis*
olivacea, *Prasinococcus capsulatus*, *Scherffelia dubia*,
Tetraselmis chui, *Tetraselmis suecica*, *Mantoniella squamata*,
Ostreococcus tauri, Rubiaceae, such as the genus *Coffea*, for
 example the genera and species *Coffea* spp., *Coffea arabica*,
 10 *Coffea canephora* or *Coffea liberica* [coffee],
 Scrophulariaceae, such as the genus *Verbascum*, for example the
 genera and species *Verbascum blattaria*, *Verbascum chaixii*,
Verbascum densiflorum, *Verbascum lagurus*, *Verbascum*
longifolium, *Verbascum lychnitis*, *Verbascum nigrum*, *Verbascum*
 15 *olympicum*, *Verbascum phlomoides*, *Verbascum phoenicum*,
Verbascum pulverulentum or *Verbascum thapsus* [verbascum],
 Solanaceae, such as the genera *Capsicum*, *Nicotiana*, *Solanum*,
Lycopersicon, for example the genera and species *Capsicum*
annuum, *Capsicum annum* var. *glabriusculum*, *Capsicum*
 20 *frutescens* [pepper], *Capsicum annum* [paprika], *Nicotiana*
tabacum, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana*
glauca, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*,
Nicotiana quadrivalvis, *Nicotiana repanda*, *Nicotiana rustica*,
Nicotiana sylvestris [tobacco], *Solanum tuberosum* [potato],
 25 *Solanum melongena* [eggplant] *Lycopersicon esculentum*,
Lycopersicon lycopersicum, *Lycopersicon pyriforme*, *Solanum*
integrifolium or *Solanum lycopersicum* [tomato], Sterculiaceae,
 such as the genus *Theobroma*, for example the genus and species
Theobroma cacao [cacao] or Theaceae, such as the genus
 30 *Camellia*, for example the genus and species *Camellia sinensis*
 [tea].

Transgenic plants such as dicotyledonous or monocotyledonous
 plants are employed in the process. Those which are especially
 35 advantageously employed in the process are oil crop plants
 which comprise large amounts of lipid compounds, such as
 peanut, oilseed rape, canola, sunflower, safflower (*Carthamus*
tinctoria), poppy, mustard, hemp, castor-oil plant, olive,

sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or arable crops such as
 5 maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species. Preferred plants according to the invention are oil crop plants such as
 10 peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as
 15 sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed or hemp.

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In principle, all genes of the fatty acid or lipid metabolism can be used in the process for the production of polyunsaturated fatty acids, advantageously in combination with the $\Delta 6$ -desaturase(s), $\Delta 6$ -elongase(s), $\Delta 5$ -desaturase(s),
 25 $\Delta 5$ -elongase(s), $\Delta 4$ -desaturase(s), $\Delta 12$ -desaturase(s) and/or $\omega 3$ -desaturase(s) [for the purposes of the present invention, the plural is understood as encompassing the singular and vice versa]. Genes of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-
 30 ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty
 35 acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously used in combination with the $\Delta 6$ -desaturase(s), $\Delta 6$ -elongase(s), $\Delta 5$ -desaturase(s), $\Delta 5$ -

elongase(s), $\Delta 4$ -desaturase(s), $\Delta 12$ -desaturase(s) and/or $\omega 3$ -desaturase(s), it being possible to use individual genes or a plurality of genes in combination.

- 5 In addition to the production directly in the organism, of the starting fatty acids for the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and an $\omega 3$ -desaturase used in the process, the fatty acids can also be fed externally. The production in the organism is preferred
 10 for reasons of economy. Preferred substrates are linoleic acid (C18:2 $\Delta 9,12$) and/or γ -linolenic acid (C18:3 $\Delta 6,9,12$).

To increase the yield in the above-described process for the production of oils and/or triglycerides with an advantageously
 15 elevated content of polyunsaturated fatty acids, it is advantageous to increase the amount of starting product for the synthesis of fatty acids; this can be achieved for example by introducing, into the organism, a nucleic acid which codes for a polypeptide with $\Delta 12$ -desaturase. The $\Delta 12$ -desaturase
 20 which is used in the process advantageously converts oleic acid (C18:1 $\Delta 9$) into linoleic acid (C18:2 $\Delta 9,12$) or C18:2 $\Delta 6,9$ into C18:3 $\Delta 6,9,12$ (= GLA). This increasingly provides the starting materials linoleic acid (C18:2 $\Delta 9,12$) and γ -linolenic acid (C18:3 $\Delta 6,9,12$) for the synthesis of the polyunsaturated fatty
 25 acids. This is particularly advantageous in oil-producing organisms such as those from the family of the Brassicaceae, such as the genus *Brassica*, for example oilseed rape; the family of the Elaeagnaceae, such as the genus *Elaeagnus*, for example the genus and species *Olea europaea*, or the family
 30 Fabaceae, such as the genus *Glycine*, for example the genus and species *Glycine max*, which are high in oleic acid. Since these organisms are only low in linoleic acid (Mikoklajczak et al., Journal of the American Oil Chemical Society, 38, 1961, 678 - 681), the use of the abovementioned $\Delta 12$ -desaturases for
 35 producing the starting material linoleic acid is advantageous.

Nucleic acids used in the process are advantageously derived from plants such as algae, for example algae of the family of

the Prasinophyceae such as the genera *Heteromastix*, *Mammella*, *Mantoniella*, *Micromonas*, *Nephroselmis*, *Ostreococcus*, *Prasinocladus*, *Prasinococcus*, *Pseudoscourfielda*, *Pycnococcus*, *Pyramimonas*, *Scherffelia* or *Tetraselmis* such as the genera and
 5 species *Heteromastix longifillis*, *Mamiella gilva*, *Mantoniella squamata*, *Micromonas pusilla*, *Nephroselmis olivacea*, *Nephroselmis pyriformis*, *Nephroselmis rotunda*, *Ostreococcus tauri*, *Ostreococcus* sp. *Prasinocladus ascus*, *Prasinocladus lubricus*, *Pycnococcus provasolii*, *Pyramimonas amyliifera*,
 10 *Pyramimonas disomata*, *Pyramimonas obovata*, *Pyramimonas orientalis*, *Pyramimonas parkeae*, *Pyramimonas spinifera*, *Pyramimonas* sp., *Tetraselmis apiculata*, *Tetraselmis carteriaformis*, *Tetraselmis chui*, *Tetraselmis convolutae*, *Tetraselmis desikacharyi*, *Tetraselmis gracilis*, *Tetraselmis hazeni*, *Tetraselmis impellucida*, *Tetraselmis inconspicua*,
 15 *Tetraselmis levis*, *Tetraselmis maculata*, *Tetraselmis marina*, *Tetraselmis striata*, *Tetraselmis subcordiformis*, *Tetraselmis suecica*, *Tetraselmis tetrabrachia*, *Tetraselmis tetrathele*, *Tetraselmis verrucosa*, *Tetraselmis verrucosa* fo. *Rubens* or
 20 *Tetraselmis* sp. The nucleic acids used are advantageously derived from algae of the genus *Ostreococcus*. Further advantageous organisms are diatoms such as *Thalassiosira*, *Phaeodactylum* or *Thraustochytrium* or fungi such as *Thraustochytrium* or *Phytophthora*.

25 In the process advantageously the abovementioned nucleic acid sequences or their derivatives or homologues which code for polypeptides which retain the enzymatic activity of the proteins code for by nucleic acid sequences, are. These
 30 sequences, individually or in combination with the nucleic acid sequences which code for $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase, are cloned into expression constructs and used for the introduction into, and expression in, plants. Owing to
 35 their construction, these expression constructs make possible an advantageous optimal synthesis of the polyunsaturated fatty acids produced in the process.

In a preferred embodiment, the process furthermore comprises the step of obtaining a cell or an intact organism which comprises the nucleic acid sequences used in the process, where the cell and/or the plant is transformed with a nucleic acid sequence which codes for the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase, a gene construct or a vector as described below, alone or in combination with further nucleic acid sequences which code for proteins of the fatty acid or lipid metabolism. In a further preferred embodiment, this process furthermore comprises the step of obtaining the oils, lipids or free fatty acids from the cell or plant. The culture can, for example, take the form of a fermentation culture, for example in the case of the cultivation of plant cells, or a greenhouse- or field-grown culture of a plant. The cell or the plant produced thus is advantageously a cell of an oil-producing organism, such as an oil crop, such as, for example, peanut, oilseed rape, canola, linseed, hemp, peanut, soybean, safflower, hemp, sunflowers or borage.

In the case of plant cells, plant tissue or plant organs, "growing" is understood as meaning, for example, the cultivation on or in a nutrient medium, or of the intact plant on or in a substrate, for example in a hydroponic culture, potting compost or on arable land.

For the purposes of the invention, "transgenic" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector comprising the nucleic acid sequence according to the invention or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

- a) the nucleic acid sequence according to the invention, or
- b) a genetic control sequence which is operably linked with

the nucleic acid sequence according to the invention, for example a promoter, or

c) (a) and (b)

5

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of
10 one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is
15 preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette - for
20 example the naturally occurring combination of the natural promoter of the nucleic acid sequences according to the invention with the corresponding $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase - becomes a transgenic expression
25 cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

30

A transgenic plant for the purposes of the invention is therefore understood as meaning, as above, that the nucleic acids used in the process are not at their natural locus in the genome of the plant, it being possible for the nucleic acids to be expressed homologously or heterologously. However,
35 as mentioned, transgenic also means that, while the nucleic acids according to the invention are at their natural position in the genome of the plant, the sequence has been modified with regard to the natural sequence, and/or that the

regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place.

Host organisms for the nucleic acids, the expression cassette or the vector used in the process are, in principle, advantageously all plants which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and/or which are suitable for the expression of recombinant genes. Examples which may be are plants such as Arabidopsis, Asteraceae such as Calendula or crop plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (*Carthamus tinctorius*) or cacao bean. Preferred plants are those which are naturally capable of synthesizing substantial amounts of oil, such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean or sunflower.

Plants for the purpose of the present invention include plant cells and certain tissues, organs and parts of plants in all their phenotypic forms such as anthers, fibers, root hairs, stalks, embryos, calli, cotyledons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant.

Transgenic plants which comprise the polyunsaturated fatty acids synthesized in the process can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated. Plants for the process are listed as meaning intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotyledons, petioles, harvested material, plant tissue,

reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue. However, the compounds produced in the process can also be isolated from the organisms, advantageously plants, in the form of their oils, fats, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by this process can be obtained by harvesting the organisms, either from the culture in which they grow, or from the field. This can be done via pressing or extraction of the plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty acids can be obtained by pressing by what is known as cold-beating or cold-pressing without applying heat. To allow for greater ease of disruption of the plant parts, specifically the seeds, they are previously comminuted, steamed or roasted. The seeds which have been pretreated in this manner can subsequently be pressed or extracted with solvents such as warm hexane. The solvent is subsequently removed again. In this manner, more than 96% of the compounds produced in the process can be isolated. Thereafter, the resulting products are processed further, i.e. refined. In this process, substances such as the plant mucilages and suspended matter are first removed again. What is known as desliming can be effected enzymatically or, for example, chemico-physically by addition of acid such as phosphoric acid. Thereafter, the free fatty acids are removed by treatment with a base, for example sodium hydroxide solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product and then dried. To remove the pigment remaining in the product, the products are subjected to bleaching, for example using filler's earth or active charcoal. At the end, the product is deodorized, for example using steam.

An embodiment is the use of the oils, lipids, the fatty acids and/or the fatty acid composition which have been produced by

the process or by mixing these oils, lipids and/or fatty acids with animal, microbial or vegetable oils, lipids or fatty acids in feedstuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids or fatty acids produced in the process can be
5 used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils.

The term "oil", "lipid" or "fat" is understood as meaning a
10 fatty acid mixture comprising unsaturated, saturated, preferably esterified, fatty acid(s), advantageously bound in triglycerides. The oil, lipid or fat is preferably high in polyunsaturated free or, advantageously, esterified fatty acid(s), in particular linoleic acid, γ -linolenic acid,
15 dihomogamma-linolenic acid, arachidonic acid, α -linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid, especially advantageously arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid. The amount of unsaturated
20 esterified fatty acids preferably amounts to approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. For the analysis, the fatty acid content can, for example, be determined by gas chromatography after converting the fatty acids into the
25 methyl esters by transesterification. The oil, lipid or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. The content of the various fatty acids in the oil or fat can vary, in
30 particular depending on the starting organism.

The polyunsaturated fatty acids with advantageously at least four double bonds which are produced in the process are, as described above, for example sphingolipids, phosphoglycerides,
35 lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol, triacylglycerol or other fatty acid esters, preferably triacylglycerides.

Starting from the polyunsaturated fatty acids with advantageously at least five or six double bonds, which acids have been prepared in the process, the polyunsaturated fatty acids which are present can be liberated for example via
5 treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification via, for example, H₂SO₄. The fatty acids can also
10 be liberated directly without the above-described processing step.

After their introduction into an organism, advantageously a plant cell or plant, the nucleic acids used in the process can
15 either be present on a separate plasmid or, advantageously, integrated into the genome of the host cell. In the case of integration into the genome, integration can be random or else be effected by recombination such that the native gene is replaced by the copy introduced, whereby the production of the
20 desired compound by the cell is modulated, or by the use of a gene in trans, so that the gene is linked operably with a functional expression unit which comprises at least one sequence which ensures the expression of a gene and at least one sequence which ensures the polyadenylation of a
25 functionally transcribed gene. The nucleic acids are advantageously introduced into the organisms via multiexpression cassettes or constructs for multiparallel expression, advantageously into the plants for the multiparallel seed-specific expression of genes.

30

Mosses and algae are the only known plant systems which produce substantial amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs
35 in membrane lipids, while algae, organisms which are related to algae and a few fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction. This is why nucleic acid molecules which are isolated from such strains which also

accumulate PUFAs in the triacylglycerol fraction are particularly advantageous for the process and thus for the modification of the lipid and PUFA production system in a host, in particular plants such as oil crop plants, for example oilseed rape, canola, linseed, hemp, soybeans, sunflowers and borage. They can therefore be used advantageously in the process.

Substrates which are advantageously suitable for the nucleic acids which are used in the process and which code for polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and $\omega 3$ -desaturase activity and/or the further nucleic acids used, such as the nucleic acids which code for polypeptides of the fatty acid or lipid metabolism selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16} -, C_{18} - or C_{20} -fatty acids. The fatty acids converted as substrates in the process are preferably converted in the form of their acyl-CoA esters and/or their phospholipid esters.

To produce the long-chain PUFAs, the polyunsaturated C_{18} -fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase. After one elongation cycle, this enzyme activity gives C_{20} -fatty acids and after two elongation cycles C_{22} -fatty acids. The activity of the desaturases and elongases used in the process preferably leads to C_{20} - and/or C_{22} -fatty acids with at least four double bonds in the fatty acid molecule, preferably to give C_{20} -fatty acids with at least four double bonds, especially preferably to give C_{20} - and/or

C₂₂-fatty acids with at least five or six double bonds, very specially preferably with six double bonds in the molecule. Produces of the process which are especially preferred are arachidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid. The C₂₀- and/or C₂₂-fatty acids with at least four double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

The preferred biosynthesis site of the fatty acids, oils, lipids or fats in the plants which are advantageously used is, for example, in general the seed or cell strata of the seed, so that seed-specific expression of the nucleic acids used in the process makes sense. However, it is obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue, but can also take place in a tissue-specific manner in all the other parts of the plant, for example in epidermal cells or in the tubers.

Owing to the use of the nucleic acids according to the invention which code for a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 12$ -desaturase and $\omega 3$ -desaturase, the polyunsaturated fatty acids produced in the process can be increased by at least 5%, preferably by at least 10%, especially preferably by at least 20%, very especially preferably by at least 50% in comparison with the wild types of the plants which do not comprise the nucleic acids recombinantly.

In principle, the polyunsaturated fatty acids produced by the process can be increased in the plants used in the process in two different ways. Advantageously, the pool of free polyunsaturated fatty acids and/or the content of the esterified polyunsaturated fatty acids produced via the process can be enlarged. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic

plants is enlarged by the process.

The fatty acids obtained in the process are also suitable as starting material for the chemical synthesis of the products of value. They can be used, for example, in combination with one another or alone for the production of pharmaceuticals, foodstuffs, animal feed or cosmetics.

A further subject matter are isolated nucleic acid sequences which code for polypeptides with $\Delta 6$ -desaturase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,
- b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 2, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which code for polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 2 and which have a $\Delta 6$ -desaturase activity.

A further subject matter are isolated nucleic acid sequences which code for polypeptides with $\Delta 6$ -elongase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7,
- b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 which code for polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and

which have a $\Delta 6$ -elongase activity.

A further subject matter are isolated nucleic acid sequences which code for polypeptides with $\Delta 5$ -desaturase activity,
5 selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 9,
- 10 b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 10, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 9 which code for polypeptides with at least 40% identity
15 at the amino acid level with SEQ ID NO: 10 and which have a $\Delta 5$ -desaturase activity.

A further subject matter are isolated nucleic acid sequences which code for polypeptides with $\omega 3$ -desaturase activity,
20 selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 23,
- 25 b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 24, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 23 which code for polypeptides with at least 40% identity
30 at the amino acid level with SEQ ID NO: 24 and which have a $\omega 3$ -desaturase activity.

The invention furthermore relates to isolated nucleic acid sequences which code for polypeptides with $\Delta 12$ -desaturase
35 activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 25, or

b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 26, or

5 c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 25 which code for polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 26 and which have a $\Delta 12$ -desaturase activity.

10 The invention furthermore relates to gene constructs which comprise the nucleic acid sequences SEQ ID NO: 25 according to the invention, wherein the nucleic acid is linked operably with one or more regulatory signals. In addition, additional biosynthesis genes of the fatty acid or lipid metabolism
15 comprises selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A
20 carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) may be present in the gene construct. Advantageously, biosynthesis genes of the fatty
25 acid or lipid metabolism selected from the group $\Delta 4$ -desaturase, $\Delta 8$ -desaturase, $\Delta 9$ -elongase or $\Delta 5$ -elongase are additionally present.

All of the nucleic acid sequences used in the process are
30 advantageously derived from an organism such as a plant or a microorganism. The nucleic acid sequences are preferably derived from algae such as *Ostreococcus*, fungi such as *Phytophthora* or from diatoms such as *Thalassiosira*.

35 The nucleic acid sequences used in the process which code for proteins with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase activity are advantageously introduced alone or, preferably,

in combination in an expression cassette (= nucleic acid construct) which makes possible the expression of the nucleic acids in a plant. The nucleic acid construct can comprise more than one nucleic acid sequence of an enzymatic activity, such as, for example, of a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase.

To introduce the nucleic acids used in the process, the latter are advantageously amplified and ligated in the known manner. Preferably, a procedure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primer(s) are selected taking into consideration the sequence to be amplified. The primers should advantageously be chosen in such a way that the amplificate comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificate is expediently analyzed. For example, a gel-electrophoretic separation can be carried out, which is followed by a quantitative and a qualitative analysis. Thereafter, the amplificate can be purified following a standard protocol (for example Qiagen). An aliquot of the purified amplificate is then available for the subsequent cloning step. Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they comprise at least the vir genes required for the Agrobacterium-mediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems advantageously also comprise further cis-regulatory regions such as promoters and terminator sequences and/or selection markers, by means of which suitably transformed organisms can be identified. While in the case of cointegrated

vector systems vir genes and T-DNA sequences are arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. Owing to this fact, the last-mentioned vectors are relatively small, easy to manipulate and to replicate both in *E. coli* and in *Agrobacterium*. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, Bin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al, Trends in Plant Science (2000) 5, 446-451. In order to prepare the vectors, the vectors can first be linearized with restriction endonuclease(s) and then modified enzymatically in a suitable manner. Thereafter, the vector is purified, and an aliquot is employed for the cloning step. In the cloning step, the enzymatically cleaved and, optionally, purified amplificate is cloned with vector fragments which have been prepared in a similar manner, using ligase. In this context, a particular nucleic acid construct, or vector or plasmid construct, can have one or else more than one codogenic gene segments. The codogenic gene segments in these constructs are preferably linked operably with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as the above-described promoters and terminator sequences. The constructs can advantageously be stably propagated in microorganisms, in particular in *E. coli* and *Agrobacterium tumefaciens*, under selective conditions and make possible the transfer of heterologous DNA into plants or microorganisms.

The nucleic acids used in the process, the inventive nucleic acids and nucleic acid constructs, can be introduced into organisms such as microorganisms or advantageously plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published and cited in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, p. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in:

Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225. Thus, the nucleic acids, the inventive nucleic acids and nucleic acid constructs, and/or vectors used in the process can be used for the recombinant modification of a broad spectrum of organisms, advantageously plants, so that the latter become better and/or more efficient PUFA producers.

A series of mechanisms by which a modification of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 12$ -desaturase and $\omega 3$ -desaturase protein and of the further proteins used in the process, such as $\Delta 5$ -elongase or $\Delta 4$ -desaturase protein, is possible exist, so that the yield, production and/or production efficiency of the advantageous polyunsaturated fatty acids in a plant, preferably in an oil crop plant, can be influenced directly owing to this modified protein. The number or activity of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and $\omega 3$ -desaturase proteins or genes can be increased, so that greater amounts of the gene products and, ultimately, greater amounts of the polyunsaturated fatty acids with at least four double bonds produced in the process are produced. A *de novo* synthesis in a plant which has lacked the activity and ability to biosynthesize the compounds prior to introduction of the corresponding gene(s) is also possible. This applies analogously to the combination with further desaturases or elongases or further enzymes of the fatty acid and lipid metabolism. The use of various divergent sequences, i.e. sequences which differ at the DNA sequence level, may also be advantageous in this context, or else the use of promoters for gene expression which make possible a different gene expression in the course of time, for example as a function of the degree of maturity of a seed or an oil-storing tissue.

Owing to the introduction of a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase gene into a plant, alone or in combination with other genes in a cell, it is not only possible to increase biosynthesis flux towards the end product, but also to increase, or to create *de novo* the corresponding triacylglycerol composition. Likewise, the number or activity of other genes which are involved in the import of nutrients which are required for the biosynthesis of one or more fatty acids, oils, polar and/or neutral lipids, can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, whereby the ability of the cells to produce PUFAs as described below is enhanced further. By optimizing the activity or increasing the number of one or more $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involved in the degradation of these compounds, an enhanced yield, production and/or efficiency of production of fatty acid and lipid molecules in plants, is made possible.

The isolated nucleic acid molecules used in the process code for proteins or parts of these, where the proteins or the individual protein or parts thereof comprise(s) an amino acid sequence with sufficient homology to an amino acid sequence which is shown in the sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26, so that the proteins or parts thereof retain $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase activity. The proteins or parts thereof which is/are code for by the nucleic acid molecule(s) preferably retain their essential enzymatic activity and the ability of participating in the metabolism of compounds required for the synthesis of cell membranes or lipid bodies

in plants, or in the transport of molecules across these membranes. Advantageously, the proteins code for by the nucleic acid molecules have at least approximately 40%, preferably at least approximately 50% or 60% and more preferably at least approximately 70%, 80% or 90% and most preferably at least approximately 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity with the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26. For the purposes of the invention, homology or homologous is understood as meaning identity or identical, respectively.

The homology was calculated over the entire amino acid or nucleic acid sequence region. The skilled worker has available a series of programs which are based on various algorithms for the comparison of various sequences. Here, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151-153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981))], which are comprised in the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program GAP and the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the sequence alignments.

Essential enzymatic activity of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase used in the process is understood as meaning that they retain at least an enzymatic

activity of at least 10%, preferably 20%, especially preferably 30% and very especially 40% in comparison with the proteins/enzymes code ford by the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 and their derivatives and can thus participate in the metabolism of compounds required for the synthesis of fatty acids, fatty acid esters such as diacylglycerides and/or triacylglycerides in a plant or a plant cell, or in the transport of molecules across membranes, meaning C₂₀- or C₂₂- carbon chains in the fatty acid molecule with double bonds at least four, five or six positions.

Nucleic acids which can advantageously be used in the process are derived from bacteria, fungi, diatoms, animals such as *Caenorhabditis* or *Oncorhynchus* or plants such as algae or mosses, such as the genera *Shewanella*, *Physcomitrella*, *Thraustochytrium*, *Fusarium*, *Phytophthora*, *Ceratodon*, *Mantoniella*, *Ostreococcus*, *Isochrysis*, *Aleurita*, *Muscarioides*, *Mortierella*, *Borago*, *Phaeodactylum*, *Crypthecodinium*, specifically from the genera and species *Oncorhynchus mykiss*, *Thalassiosira pseudonona*, *Mantoniella squamata*, *Ostreococcus* sp., *Ostreococcus tauri*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Phytophthora sojae*, *Fusarium gramineum*, *Cryptocodium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Muscarioides viallii*, *Mortierella alpina*, *Borago officinalis*, *Phaeodactylum tricornutum*, *Caenorhabditis elegans* or particularly advantageously from *Oncorhynchus mykiss*, *Thalassiosira pseudonona* or *Crypthecodinium cohnii*.

Alternatively, nucleotide sequences which code for a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase and which advantageously hybridize under stringent conditions with a nucleotide sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ

ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 can be used in the process.

- 5 The nucleic acid sequences used in the process are advantageously introduced into an expression cassette which makes possible the expression of the nucleic acids in organisms such as microorganisms or plants.
- 10 In doing so, the nucleic acid sequences which code for $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase are linked operably with one or more regulatory signals, advantageously for enhancing gene expression. These regulatory sequences are
- 15 intended to make possible the specific expression of the genes and proteins. Depending on the host organism, this may mean, for example, that the gene is expressed and/or overexpressed only after induction has taken place, or else that it is expressed and/or overexpressed immediately. For example, these
- 20 regulatory sequences take the form of sequences to which inductors or repressors bind, thus controlling the expression of the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the
- 25 actual structural genes and, optionally, may have been genetically modified in such a way that their natural regulation is eliminated and the expression of the genes is enhanced. However, the expression cassette (= expression construct = gene construct) can also be simpler in
- 30 construction, that is to say no additional regulatory signals have been inserted before the nucleic acid sequence or its derivatives, and the natural promoter together with its regulation was not removed. Instead, the natural regulatory sequence has been mutated in such a way that regulation no
- 35 longer takes place and/or gene expression is enhanced. These modified promoters can also be positioned on their own before the natural gene in the form of part-sequences (= promoter with parts of the nucleic acid sequences used in accordance

with the invention) in order to enhance the activity. Moreover, the gene construct may advantageously also comprise one or more what are known as enhancer sequences in operable linkage with the promoter, which make possible an enhanced
5 expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminators, may also be inserted at the 3' end of the DNA sequences. The $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase
10 genes may be present in one or more copies of the expression cassette (= gene construct). Preferably, only one copy of the genes is present in each expression cassette. This gene construct or the gene constructs can be expressed together in the host organism. In this context, the gene construct(s) can
15 be inserted in one or more vectors and be present in the cell in free form, or else be inserted in the genome. It is advantageous for the insertion of further genes in the host genome when the genes to be expressed are present together in one gene construct.

20 In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes introduced, thus enhancing it. Thus, an enhancement of the regulatory elements, advantageously at
25 the transcriptional level, may take place by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhanced translation is also possible, for example by improving the stability of the mRNA.

30 A further embodiment of the invention is one or more gene constructs. Gene constructs may comprise one or more sequences which are defined by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID
35 NO: 23 or SEQ ID NO: 25 or its derivatives and which code for polypeptides as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID

NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26. The abovementioned $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase proteins lead advantageously to a desaturation or elongation of fatty acids, the substrate advantageously having one, two, three, four, five or six double bonds and advantageously 18, 20 or 22 carbon atoms in the fatty acid molecule. The same applies to their homologs, derivatives or analogs, which are linked operably with one or more regulatory signals, advantageously for enhancing gene expression.

Advantageous regulatory sequences which can be employed for the preparation of the nucleic acid sequences employed in the process and their subsequent introduction into plants are present for example in promoters such as the cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacIq, T7, T5, T3, gal, trc, ara, SP6, λ -PR or λ -PL promoter and are advantageously employed in Gram-negative bacteria. Further advantageous regulator sequences are, for example, present in the Gram-positive promoters amy and SP02, in the yeast or fungal promoters ADC1, MF α , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH or in the plant promoters CaMV/35S [Franck et al., Cell 21 (1980) 285-294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, nos or in the ubiquitin or phaseolin promoter. Advantageous in this context are also inducible promoters, such as the promoters described in EP-A-0 388 186 (benzenesulfonamide-inducible), Plant J. 2, 1992:397-404 (Gatz et al., tetracycline-inducible), EP-A-0 335 528 (abscissic acid-inducible) or WO 93/21334 (ethanol- or cyclohexenol-inducible) promoters. Further suitable plant promoters are the cytosolic FBPase promoter or the ST-LSI promoter of potato (Stockhaus et al., EMBO J. 8, 1989, 2445), the glycine max phosphoribosylpyrophosphate amidotransferase promoter (Genbank Accession No. U87999) or the node-specific promoter described in EP-A-0 249 676. Especially advantageous promoters are promoters which make possible the expression in tissues which are involved in the biosynthesis of fatty acids. Very especially advantageous are seed-specific promoters, such

as the USP promoter as described, but also other promoters such as the LeB4, DC3, phaseolin or napin promoter. Further especially advantageous promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (oilseed rape napin promoter), WO 98/45461 (Arabidopsis oleosin promoter), US 5,504,200 (Phaseolus vulgaris phaseolin promoter), WO 91/13980 (Brassica Bce4 promoter), by Bäumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. Examples of promoters which are suitable for monocots are the barley lpt-2 or lpt-1 promoter (WO 95/15389 and WO 95/23230), the barley hordein promoter and other suitable promoters described in WO 99/16890.

In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. It is also possible and advantageous to use synthetic promoters, either in addition or alone, in particular when they mediate seed-specific expression, such as those described in WO 99/16890.

In order to achieve a particularly high PUFA content, before in transgenic plants, the PUFA biosynthesis genes should advantageously be expressed in oil crops in a seed-specific manner. To this end, seed-specific promoters can be used, or those promoters which are active in the embryo and/or in the endosperm. In principle, seed-specific promoters can be isolated both from dicotyledonous and from monocotyledonous plants. Advantageous preferred promoters are listed hereinbelow: USP (= unknown seed protein) and vicilin (Vicia faba) [Bäumlein et al., Mol. Gen Genet., 1991, 225(3)], napin (oilseed rape) [US 5,608,152], acyl carrier protein (oilseed rape) [US 5,315,001 and WO 92/18634], oleosin (Arabidopsis thaliana) [WO 98/45461 and WO 93/20216], phaseolin (Phaseolus vulgaris) [US 5,504,200], Bce4 [WO 91/13980], leguminous B4 (LegB4 promoter) [Bäumlein et al., Plant J., 2,2, 1992], Lpt2 and lpt1 (barley) [WO 95/15389 and WO95/23230], seed-specific

promoters from rice, maize and wheat [WO 99/16890], Amy32b, Amy 6-6 and aleurain [US 5,677,474], Bce4 (oilseed rape) [US 5,530,149], glycinin (soybean) [EP 571 741], phosphoenolpyruvate carboxylase (soybean) [JP 06/62870], ADR12-2
5 (soybean) [WO 98/08962], isocitrate lyase (oilseed rape) [US 5,689,040] or α -amylase (barley) [EP 781 849].

Plant gene expression can also be facilitated via a chemically inducible promoter (see review in Gatz 1997, Annu. Rev. Plant
10 Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that gene expression should take place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracycline-inducible promoter
15 (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations,
20 each of the nucleic acids which code for $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase and which are used in the process should be expressed under the control of a separate promoter, preferably a promoter which differs from the other
25 promoters, since repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site, advantageously in a polylinker, for insertion
30 of the nucleic acid to be expressed and, optionally, a terminator sequence is positioned behind the polylinker. This sequence is repeated several times, preferably three, four or five times, so that up to five genes can be combined in one construct and introduced into the transgenic plant in order to
35 be expressed. Advantageously, the sequence is repeated up to three times, or as many times as required. To express the nucleic acid sequences, the latter are inserted behind the promoter via a suitable cleavage site, for example in the

polylinker. Advantageously, each nucleic acid sequence has its own promoter and, optionally, its own terminator sequence. Such advantageous constructs are disclosed, for example, in DE 101 02 337 or DE 101 02 338. However, it is also possible to
5 insert a plurality of nucleic acid sequences behind a promoter and, optionally, before a terminator sequence. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be inserted at the first
10 or last position in the cassette without its expression being substantially influenced thereby. Advantageously, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminator sequences can be used in the expression cassette. However, it is also possible to use
15 only one type of promoter in the cassette. This, however, may lead to undesired recombination events.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by
20 suitable terminator sequences at the 3' end of the biosynthesis genes which have been introduced (behind the stop codon). An example of a sequence which can be used in this context is the OCS 1 terminator sequence. As is the case with the promoters, different terminator sequences should be used
25 for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host plants,
30 and to express therein, regulatory genes such as genes for inducers, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the
35 fatty acid or lipid metabolism can advantageously be present in a nucleic acid construct, or gene construct; however, these genes can also be positioned on one or more further nucleic acid constructs. Biosynthesis genes of the fatty acid or lipid

metabolism which are advantageously used is a gene selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allene oxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s) or combinations thereof. Especially advantageous nucleic acid sequences are biosynthesis genes of the fatty acid or lipid metabolism selected from the group of the acyl-CoA:lysophospholipid acyltransferase, $\Delta 4$ -desaturase, $\Delta 8$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 9$ -elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression cassettes, like those mentioned above, in combination with other elongases and desaturases and used for transforming plants with the aid of *Agrobacterium*.

Here, the regulatory sequences or factors can, as described above, preferably have a positive effect on, and thus enhance, the expression of the genes which have been introduced. Thus, enhancement of the regulatory elements can advantageously take place at the transcriptional level by using strong transcription signals such as promoters and/or enhancers. However, an enhanced translation is also possible, for example by improving the stability of the mRNA. In principle, the expression cassettes can be used directly for introduction into the plants or else be introduced into a vector.

These advantageous vectors, preferably expression vectors, comprise the nucleic acids which code for the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase and which are used in the process, or else a nucleic acid construct which the nucleic acid used either alone or in combination with further biosynthesis genes of the fatty acid or lipid metabolism such

as the acyl-CoA:lysophospholipid acyltransferases, $\Delta 4$ -desaturase, $\Delta 8$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 9$ -elongase. As used in the present context, the term "vector" refers to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced (for example bacterial vectors with bacterial replication origin). Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used exchangeably since the plasmid is the form of vector which is most frequently used. However, the invention is also intended to cover other forms of expression vectors, such as viral vectors, which exert similar functions. Furthermore, the term "vector" is also intended to encompass other vectors with which the skilled worker is familiar, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors advantageously used in the process comprise the nucleic acids described below or the above-described gene construct in a form which is suitable for expressing the nucleic acids used in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences, selected on the basis of the host cells used for the expression, which regulatory sequence(s) is/are linked operably with the nucleic acid sequence to be

expressed. In a recombinant expression vector, "linked operably" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is possible and they are bound to each other in such a way that both sequences carry out the predicted function which is ascribed to the sequence (for example in an in-vitro transcription/translation system, or in a host cell if the vector is introduced into the host cell). The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Florida, Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of host cell to be transformed, the expression level of the desired protein and the like.

The recombinant expression vectors used can be designed for the expression of $\Delta 6$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\Delta 5$ -elongases, $\Delta 4$ -desaturases, $\Delta 12$ -desaturases and/or $\omega 3$ -desaturases in prokaryotic or eukaryotic cells. This is advantageous since intermediate steps of the vector construction are frequently carried out in microorganisms for the sake of simplicity. For example, the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase genes can be expressed in bacterial cells, insect cells (using Baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A., et al. (1992) "Foreign gene expression in yeast: a review",

Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Ed., pp. 1-28, Cambridge University Press: Cambridge), algae (Falciatore et al., 1999, Marine Biotechnology.1, 3:239-251), using vectors in a transformation method as described in WO 98/01572 and, preferably, in cells of multi-celled plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants" Plant Cell Rep.:583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, Chapter 6/7, pp. 71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225 (and references cited therein)). Suitable host cells are furthermore discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). As an alternative, the recombinant expression vector can be transcribed and translated in vitro, for example using T7-promoter regulatory sequences and T7-polymerase.

In most cases, the expression of proteins in prokaryotes involves the use of vectors comprising constitutive or inducible promoters which govern the expression of fusion or nonfusion proteins. Typical fusion expression vectors are, inter alia, pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) und pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose-E binding protein and protein A, respectively, is fused with the recombinant target protein.

Examples of suitable inducible nonfusion E. coli expression vectors are, inter alia, pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). The target gene expression from the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by the host RNA polymerase. The target gene expression from the vector pET 11d is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a viral RNA polymerase (T7 gn1), which is coexpressed. This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

Other vectors which are suitable for prokaryotic organisms are known to the skilled worker, these vectors are, for example in E. coli pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M13mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λ gt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples for vectors for expression in the yeast S. cerevisiae comprise pYeDesaturase1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi [J.W. Bennet & L.L. Lasure, Ed.,

pp. 396-428: Academic Press: San Diego]. Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLye23.

5 As an alternative, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase can be expressed in insect cells using Baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for
10 example Sf9 cells) comprise the pAc series (Smith et al. (1983) Mol. Cell Biol.. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The abovementioned vectors are only a small overview over
15 suitable vectors which are possible. Further plasmids are known to the skilled worker and are described, for example, in: Cloning Vectors (Ed. Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and
20 eukaryotic cells, see the Chapters 16 and 17 in Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

25 Finally, the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase gene can be expressed in plant cells or intact plants (for example spermatophytes such as arable crops). Examples of
30 plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) "Binary Agrobacterium
35 vectors for plant transformation", Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and which are linked operably so that
5 each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984) 835
10 et seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminator sequences which are functionally active in plants are also suitable.

Since plant gene expression is very often not limited to the
15 transcriptional level, a plant expression cassette preferably comprises other sequences which are linked operably, such as translation enhancers, for example the overdrive sequence, which enhances the tobacco mosaic virus 5' - untranslated leader sequence, which increases the protein/RNA ratio (Gallie
20 et al., 1987, Nucl. Acids Research 15:8693-8711).

As described above, plant gene expression must be linked operably with a suitable promoter which triggers gene expression with the correct timing or in a cell- or tissue-
25 specific manner. Utilizable promoters are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the
30 promoter of the small Rubisco subunit, which is described in US 4,962,028.

Other preferred sequences for use in operable linkage in plant gene expression cassettes are targeting sequences, which are
35 required for steering the gene product into its corresponding cell compartment (see a review in Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423 and references cited therein), for example into the vacuole, into the nucleus, all types of

plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, elaioplasts, peroxisomes and other compartments of plant cells.

5

As described above, plant gene expression can also be facilitated via a chemically inducible promoter (see review in Gatz 1997, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48:89-108). Chemically inducible promoters are particularly suitable
10 when it is desired that the gene expression takes place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) *Plant J.* 2, 397-404) and an ethanol-inducible promoter.

15

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., *Plant. Mol. Biol.* 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the
20 chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Especially preferred are those promoters which bring about the gene expression in tissues and organs in which the
25 biosynthesis of fatty acids, lipids and oils takes place, in seed cells, such as cells of the endosperm and of the developing embryo. Suitable promoters are the oilseed rape napin gene promoter (US 5,608,152), the *Vicia faba* USP promoter (Baeumlein et al., *Mol Gen Genet*, 1991, 225 (3):459-
30 67), the *Arabidopsis* oleosin promoter (WO 98/45461), the *Phaseolus vulgaris* phaseolin promoter (US 5,504,200), the *Brassica Bce4* promoter (WO 91/13980) or the legumine B4 promoter (LeB4; Baeumlein et al., 1992, *Plant Journal*, 2 (2):233-9), and promoters which bring about the seed-specific
35 expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable noteworthy promoters are the barley lpt2 or lpt1 gene promoter (WO 95/15389 and WO 95/23230) or the promoters from the barley hordein gene,

the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, the wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene or the rye secalin gene, which are described in
5 WO 99/16890.

In particular, it may be desired to bring about the multiparallel expression of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase
10 and/or $\omega 3$ -desaturase used in the process. Such expression cassettes can be introduced via a simultaneous transformation of a plurality of individual expression constructs or, preferably, by combining a plurality of expression cassettes on one construct. Also, a plurality of vectors can be
15 transformed with in each case a plurality of expression cassettes and then transferred into the host cell.

Other promoters which are likewise especially suitable are those which bring about a plastid-specific expression, since
20 plastids constitute the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters, such as the viral RNA polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the clpP promoter from Arabidopsis, described in WO 99/46394.

25 Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context,
30 are intended to comprise a multiplicity of methods known in the prior art for the introduction of foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence,
35 chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory

Manual., 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory textbooks such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey.

Host cells which are suitable in principle for taking up the nucleic acid according to the invention, the gene product according to the invention or the vector according to the invention are all prokaryotic or eukaryotic organisms. The host organisms which are advantageously used as intermediate hosts are microorganisms such as fungi or yeasts. Plants such as oil crop plants, which are high in lipid compounds, such as oilseed rape, evening primrose, hemp, thistle, peanut, canola, linseed, soybean, safflower, sunflower, borage, or plants such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanacea plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut), and fodder crops are preferably used in the process. Especially preferred plants according to the invention are oil crop plants such as soybean, peanut, oilseed rape, canola, linseed, hemp, evening primrose, sunflower, safflower, trees (oil palm, coconut).

A further subject matter are the nucleic acid sequences which are enumerated hereinbelow and which code for $\Delta 6$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\omega 3$ -desaturases and/or $\Delta 12$ -desaturases.

Isolated nucleic acid sequences which code for polypeptides with $\Delta 6$ -desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which code for a protein with the

sequence shown in SEQ ID NO: 2, or

- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which code for polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 2 and have a $\Delta 6$ -desaturase activity.

Isolated nucleic acid sequences which code for polypeptides with $\Delta 6$ -elongase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7,
- b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or

- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 which code for polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and which have a $\Delta 6$ -elongase activity.

Isolated nucleic acid sequences which code for polypeptides with $\Delta 5$ -desaturase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 9,

b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 10, or

- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 9 which code for polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 10 and which have a $\Delta 5$ -desaturase activity.

Isolated nucleic acid sequences which code for polypeptides with ω 3-desaturase activity, selected from the group consisting of:

5

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 23,

10

b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 24, or

15

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 23 which code for polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 24 and which have a ω 3-desaturase activity.

Isolated nucleic acid sequences which code for polypeptides with Δ 12-desaturase activity, selected from the group consisting of:

20

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 25, or

25

b) nucleic acid sequences which code for a protein with the sequence shown in SEQ ID NO: 26, or

30

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 25 which code for polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 26 and which have a Δ 12-desaturase activity.

The abovementioned nucleic acids are derived from organisms such as fungi, plants such as algae or diatoms which are capable of synthesizing PUFAs.

35

The isolated abovementioned nucleic acid sequences are advantageously derived from the diatom genus *Thalassiosira* or from the family *Prasinophyceae* such as the genus *Ostreococcus*,

from the class Euglenophyceae such as the genus *Euglenia* or Pythiaceae such as the genus *Phytophthora*.

Especially preferred nucleic acid sequences are derived from the genus and species *Phytophthora sojae*. In an advantageous embodiment, these nucleic acid sequences make possible the synthesis of EPA and/or ARA. The table which follows represents such advantageous nucleic acid sequences.

Gene	Enzyme function	Protein sequence	SEQ ID
D6-Des (Ps)	$\Delta 6$ -desaturase	456 As	SEQ ID NO: 1
D6-Elo (Ps)	$\Delta 6$ -elongase	304 As	SEQ ID NO: 3
D6-Elo (Ps) 2	$\Delta 6$ -elongase	278 As	SEQ ID NO: 5
D6-Elo (Ps) 3	$\Delta 6$ -elongase	278 As	SEQ ID NO: 7
D5-Des (Ps)	$\Delta 5$ -desaturase	498 As	SEQ ID NO: 9
D12-Des (Ps)	$\Delta 12$ -desaturase	398 As	SEQ ID NO: 25
O3-Des (Ps)	$\omega 3$ -desaturase	363 As	SEQ ID NO: 23

10

As described above, a further subject matter are isolated nucleic acid sequences which code for polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase and $\Delta 12$ -desaturase activity where the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase and/or $\Delta 12$ -desaturase code for by these nucleic acid sequences convert C₁₈- or C₂₀-fatty acids with one, two, three or four double bonds such as C₁₈:1 ^{$\Delta 9$} , C₁₈:2 ^{$\Delta 9,12$} or C₁₈:3 ^{$\Delta 9,12,15$} polyunsaturated C₂₀-fatty acids with three or four double bonds such as C₂₀:3 ^{$\Delta 8,11,14$} or C₂₀:4 ^{$\Delta 8,11,14,17$} . The fatty acids are advantageously desaturated in the phospholipids or CoA-fatty acid esters, advantageously in the CoA-fatty acid esters.

In an advantageous embodiment, the term "nucleic acid (molecule)" as used in the present context additionally comprises the untranslated sequence at the 3' and at the 5' end of the coding gene region: at least 500, preferably 200,

25

especially preferably 100 nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, especially preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region.

5 An "isolated" nucleic acid molecule is separate from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid is
10 derived (for example sequences which are located at the 5' and 3' ends of the nucleic acid). In various embodiments, the isolated $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase and $\Delta 12$ -desaturase molecule can comprise for example fewer than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb,
15 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived.

The nucleic acid molecules used in the process, for example a
20 nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 or of a part thereof can be isolated using molecular-biological
25 standard techniques and the sequence information provided herein. Also, for example a homologous sequence or homologous, conserved sequence regions can be identified at the DNA or amino acid level with the aid of comparative algorithms. They can be used as hybridization probe or standard hybridization
30 techniques (such as, for example, those described in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences which can be used in the process. Moreover, a
35 nucleic acid molecule comprising a complete sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25

or a part thereof can be isolated by polymerase chain reaction, where oligonucleotide primers which on the basis of this sequence or on parts thereof are used (for example a nucleic acid molecule comprising the complete sequence or part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been generated based on this same sequence). For example, mRNA can be isolated from cells (for example by means of the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA by means of reverse transcriptase (for example Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for the amplification by means of polymerase chain reaction can be generated based on one of the sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 with the aid of the amino acid sequences detailed in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26. A nucleic acid according to the invention can be amplified by standard PCR amplification techniques using cDNA or, alternatively, genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified thus can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides which correspond to a desaturase nucleotide sequence can be generated by standard synthetic methods, for example using an automatic DNA synthesizer.

Homologs of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase nucleic acid sequences with the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 means, for

example, allelic variants with at least approximately 40 or 50%, preferably at least approximately 60 or 70%, more preferably at least approximately 70 or 80%, 90% or 95% and even more preferably at least approximately 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95 %, 96%, 97%, 98%, 99% or more identity or homology with a nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 or its homologs, derivatives or analogs or parts thereof. Furthermore, isolated nucleic acid molecules of a nucleotide sequence which hybridize with a nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 or with a part thereof, for example hybridized under stringent conditions. A part thereof is understood as meaning, in accordance with the invention, that at least 25 base pairs (= bp), 50 bp, 75 bp, 100 bp, 125 bp or 150 bp, preferably at least 175 bp, 200 bp, 225 bp, 250 bp, 275 bp or 300 bp, especially preferably 350 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for the hybridization. It is also possible and advantageous to use the full sequence. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from/into the sequence detailed in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 it being intended, however, that the enzyme activity of the resulting proteins which are synthesized is advantageously retained for the insertion of one or more genes. Proteins which retain the enzymatic activity of $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase, i.e. whose activity is essentially not reduced, means proteins with at least 10%, preferably 20%, especially preferably 30%, very especially preferably 40% of the original enzyme activity in comparison with the protein code for by

SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25. The homology was calculated over the entire amino acid or nucleic acid sequence region. The skilled worker has available a series of programs which are based on various algorithms for the comparison of various sequences. Here, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151-153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981))], which are comprised in the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program GAP and the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the sequence alignments.

Homologs of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 means for example also bacterial, fungal and plant homologs, truncated sequences, single-stranded DNA or RNA of the coding and noncoding DNA sequence.

Homologs of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 also means derivatives such as, for example, promoter variants. The promoters upstream of the nucleotide sequences detailed can be modified by one or more nucleotide exchanges, by insertion(s) and/or deletion(s) without the functionality or activity of the promoters being adversely

affected, however. It is furthermore possible that the modification of the promoter sequence enhances their activity or that they are replaced entirely by more active promoters, including those from heterologous organisms.

5

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Usual lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase or into acetyl-ACP by acetyl transacylase. After condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted via a series of condensation, reduction and dehydratization reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of molecular oxygen or anaerobically (regarding the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) *E. coli* and *Salmonella*. ASM Press: Washington, D.C., pp. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) *Biology of Prokaryotes*. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) *Microbiological Reviews* 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This is made possible by acyl-CoA:lysophospholipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. Optionally, this reaction sequence can be followed repeatedly.

35

Examples of precursors for the biosynthesis of PUFAs are oleic acid, linoleic acid and linolenic acid. The C₁₈-carbon fatty acids must be elongated to C₂₀ and C₂₂ in order to obtain fatty

acids of the eicosa and docosa chain type. With the aid of the desaturases and/or elongases used in the process, arachidonic acid, eicosapentaenoic acid, and/or docosahexaenoic acid, advantageously eicosapentaenoic acid and/or docosahexaenoic acid, can be produced and subsequently employed in various applications regarding foodstuffs, feedstuffs, cosmetics or pharmaceuticals. C₂₀- and/or C₂₂-fatty acids with at least two, advantageously at least four, five or six, double bonds in the fatty acid molecule, preferably C₂₀- or C₂₂-fatty acids with advantageously five or six double bonds in the fatty acid molecule, can be prepared using the abovementioned enzymes. Substrates of the desaturases and elongases used in the process are C₁₆-, C₁₈- or C₂₀-fatty acids such as, for example, linoleic acid, γ -linolenic acid, α -linolenic acid, dihomo- γ -linolenic acid, eicosatetraenoic acid or stearidonic acid. Preferred substrates are linoleic acid, γ -linolenic acid and/or α -linolenic acid, dihomo- γ -linolenic acid, arachidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. The synthesized C₂₀- or C₂₂-fatty acids with at least four, five or six double bonds in the fatty acids are obtained in the process in the form of the free fatty acid or in the form of their esters, for example in the form of their glycerides, advantageously their triglycerides.

The term "glyceride" is understood as meaning glycerol esterified with one, two or three carboxyl radicals (mono-, di- or triglyceride). "Glyceride" is also understood as meaning a mixture of various glycerides. The glyceride or glyceride mixture may comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

For the purposes of the process, a "glyceride" is furthermore understood as meaning glycerol derivatives. In addition to the above-described fatty acid glycerides, these also include glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned in this context are the glycerophospholipids such as lecithin (phosphatidylcholine),

cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Furthermore, fatty acids must subsequently be translocated to various modification sites and incorporated into the triacylglycerol storage lipid. A further important step in lipid synthesis is the transfer of fatty acids to the polar head groups, for example by glycerol fatty acid acyltransferase (see Frentzen, 1998, *Lipid*, 100(4-5):161-166).

Publications on plant fatty acid biosynthesis and on the desaturation, the lipid metabolism and the membrane transport of lipidic compounds, on beta-oxidation, fatty acid modification and cofactors, triacylglycerol storage and triacylglycerol assembly, including the references therein, see the following papers: Kinney, 1997, *Genetic Engineering*, Ed.: JK Setlow, 19:149-166; Ohlrogge and Browse, 1995, *Plant Cell* 7:957-970; Shanklin and Cahoon, 1998, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:611-641; Voelker, 1996, *Genetic Engineering*, Ed.: JK Setlow, 18:111-13; Gerhardt, 1992, *Prog. Lipid R.* 31:397-417; Gühnemann-Schäfer & Kindl, 1995, *Biochim. Biophys Acta* 1256:181-186; Kunau et al., 1995, *Prog. Lipid Res.* 34:267-342; Stymne et al., 1993, in: *Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants*, Ed.: Murata and Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, *Plant Journal*. 13(1):1-16.

The PUFAs produced in the process comprise a group of molecules which higher animals are no longer capable of synthesizing and must therefore take up, or which higher animals are no longer capable of synthesizing themselves in sufficient quantity and must therefore take up additional quantities, although they can be synthesized readily by other organisms such as bacteria; for example, cats are no longer capable of synthesizing arachidonic acid.

Phospholipids for the purposes of the invention are understood

as meaning phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and/or phosphatidylinositol, advantageously phosphatidylcholine. The terms production or productivity are known in the art and encompass the productivity within a plant cell or a plant, that is to say the content of the desired fatty acids produced in the process relative to the content of all fatty acids in this cell or plant. The terms biosynthesis or biosynthetic pathway are known in the art and comprise the synthesis of a compound, preferably an organic compound, by a cell from intermediates, for example in a multi-step and strongly regulated process. The terms catabolism or catabolic pathway are known in the art and comprise the cleavage of a compound, preferably of an organic compound, by a cell to give catabolites (in more general terms, smaller or less complex molecules), for example in a multi-step and strongly regulated process. The term metabolism is known in the art and comprises the totality of the biochemical reactions which take place in an organism. The metabolism of a certain compound (for example the metabolism of a fatty acid) thus comprises the totality of the biosynthetic pathways, modification pathways and catabolic pathways of this compound in the cell which relate to this compound.

In a further embodiment, derivatives of the nucleic acid molecule represented in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 23 or SEQ ID NO: 25 code for proteins with at least 40%, advantageously approximately 50 or 60%, advantageously at least approximately 60 or 70% and more preferably at least approximately 70 or 80%, 80 to 90%, 90 to 95% and most preferably at least approximately 96%, 97%, 98%, 99% or more homology (= identity for the purpose of the invention) with a complete amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 24 or SEQ ID NO: 26. The homology was calculated over the entire amino acid or nucleic acid sequence region. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151-153) or

the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981))], which are comprised in the GCG software packet [Genetics Computer Group, 575 Science Drive, 5 Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program BestFit and the following settings: Gap Weight: 50, Length Weight: 3, Average 10 Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the sequence alignments.

Moreover, the invention comprises nucleic acid molecules which 15 differ from one of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 23 or SEQ ID NO: 25 (and parts thereof) owing to the degeneracy of the genetic code and which thus code for the same $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase 20 or $\Delta 12$ -desaturase activity as those code for by the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 23 or SEQ ID NO: 25.

25 In addition to the $\Delta 6$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\omega 3$ -desaturases or $\Delta 12$ -desaturases shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 23 or SEQ ID NO: 25 the skilled worker will recognize that DNA sequence polymorphisms which lead to 30 changes in the amino acid sequences of the $\Delta 6$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\omega 3$ -desaturases and/or $\Delta 12$ -desaturases may exist within a population. These genetic polymorphisms in the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase and/or $\Delta 12$ -desaturase gene may exist 35 between individuals within a population owing to natural variation. These natural variants usually bring about a variance of 1 to 5% in the nucleotide sequence of the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\omega 3$ -desaturase and/or

Δ 12-desaturase gene. Each and every one of these nucleotide variations and resulting amino acid polymorphisms in the Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase, ω 3-desaturase and/or Δ 12-desaturase which are the result of natural variation and do not modify the functional activity are to be encompassed by the invention.

Owing to their homology to the Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase, Δ 12-desaturase and/or ω 3-desaturase nucleic acids disclosed here, nucleic acid molecules which are advantageous for the process can be isolated following standard hybridization techniques under stringent hybridization conditions, using the sequences or part thereof as hybridization probe. In this context it is possible, for example, to use isolated nucleic acid molecules which are at least 15 nucleotides in length and which hybridize under stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25. Nucleic acids with at least 25, 50, 100, 250 or more nucleotides can also be used. The term "hybridizes under stringent conditions" as used in the present context is intended to describe hybridization and washing conditions under which nucleotide sequences with at least 60% homology to one another usually remain hybridized with one another. Conditions are preferably such that sequences with at least approximately 65%, preferably at least approximately 70% and especially preferably at least approximately 75% or more homology to one another usually remain hybridized to one another. These stringent conditions are known to the skilled worker and described in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred nonlimiting example of stringent hybridization conditions is hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more washing steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows

that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, regarding temperature and buffer concentration. Under "standard hybridization conditions", for example, the hybridization temperature is, depending on the type of nucleic acid, between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvents, for example 50% formamide, are present in the abovementioned buffer, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids, for example, are preferably 0.1 x SSC and 20°C to 45°C, preferably 30°C to 45°C. The hybridization conditions for DNA:RNA hybrids are, for example, preferably 0.1 x SSC and 30°C to 55°C, preferably 45°C to 55°C. The abovementioned hybridization conditions are determined by way of example for a nucleic acid with approximately 100 bp (= base pairs) in length and with a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the required hybridization conditions on the basis of the abovementioned textbooks or textbooks such as Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

In order to determine the percentage of homology (= identity) of two amino acid sequences (for example one of the sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26) or of two nucleic acids (for example SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25) the sequences are written one under the other for an optimal comparison (for example, gaps may be introduced into the

sequence of a protein or of a nucleic acid in order to generate an optimal alignment with the other protein or the other nucleic acid). Then, the amino acid residue or nucleotides at the corresponding amino acid positions or
 5 nucleotide positions are compared. If a position in a sequence is occupied by the same amino acid residue or the same nucleotide as the corresponding position in the other sequence, then the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the
 10 present context corresponds to amino acid or nucleic acid "identity"). The percentage of homology between the two sequences is a function of the number of identical positions which the sequences share (i.e. % homology = number of identical positions/total number of positions x 100). The
 15 terms homology and identity are therefore to be considered as synonymous. The programs and algorithms used are described above.

An isolated nucleic acid molecule which codes for a $\Delta 6$ -
 20 desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase which is homologous to a protein sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20,
 25 SEQ ID NO: 22, SEQ ID NO: 24 or SEQ ID NO: 26 can be generated by introducing one or more nucleotide substitutions, additions or deletions in/into a nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID
 30 NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 so that one or more amino acid substitutions, additions or deletions are introduced in/into the protein which is code ford. Mutations in one of the sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11,
 35 SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25 can be introduced by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. It is preferred to

generate conservative amino acid substitutions in one or more of the predicted nonessential amino acid residues. In a "conservative amino acid substitution", the amino acid residue is replaced by an amino acid residue with a similar side chain. Families of amino acid residues with similar side chains have been defined in the art. These families comprise amino acids with basic side chains (for example lysine, arginine, histidine), acidic side chains (for example aspartic acid, glutamic acid), uncharged polar side chains (for example glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), unpolar side chains (for example alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (for example threonine, valine, isoleucine) and aromatic side chains (for example tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase or $\omega 3$ -desaturase is thus preferably replaced by another amino acid residue from the same family of side chains. In another embodiment, the mutations can, alternatively, be introduced randomly over all or part of the sequence encoding the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase or $\omega 3$ -desaturase, for example by saturation mutagenesis, and the resulting mutants can be screened for the herein-described $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase activity in order to identify mutants which have retained the $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase, $\Delta 12$ -desaturase and/or $\omega 3$ -desaturase activity. Following the mutagenesis of one of the sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23 or SEQ ID NO: 25, the protein which is code for can be expressed recombinantly, and the activity of the protein can be determined, for example using the tests described in the present text.

The invention furthermore relates to a transgenic nonhuman organism, preferably a transgenic plant, which comprise the nucleic acid SEQ ID NO: 25 according to the invention or a gene construct or a vector which comprise these nucleic acid sequences according to the invention.

The present invention is illustrated in greater detail by the examples which follow, which are not to be construed as limiting.

Examples

Example 1: General cloning methods:

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of *E. coli* cells, bacterial cultures and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA:

Recombinant DNA molecules were sequenced with an ABI laser fluorescence DNA sequencer by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467). Fragments obtained by polymerase chain reaction were sequenced and verified to avoid polymerase errors in constructs to be expressed.

Example 3: Cloning of PUFA-specific desaturases and elongases from *Phytophthora sojae*

In order to search for novel genes with $\Delta 6$ -, $\Delta 5$ -, $\Delta 12$ -, $\omega 3$ -desaturase and $\Delta 6$ -elongase activity, a genomic database of *Phytophthora sojae* (<http://genome.jgi->

psf.org/sojae/sojae1.home.html). This database contains crude sequences which cover approximately 90% of the genomic DNA of the organism. Putative candidate genes were found for all the searched-for activities. By preparing and characterizing cDNA
 5 of these candidate genes, their correct coding open reading frames were found and the amino acid sequence was derived. It is as follows:

Gene	Enzyme function	Protein sequence	SEQ ID
D6-Des (Ps)	Δ 6-desaturase	456 As	SEQ ID NO: 1
D6-Elo (Ps)	Δ 6-elongase	304 As	SEQ ID NO: 3
D6-Elo (Ps) 2	Δ 6-elongase	278 As	SEQ ID NO: 5
D6-Elo (Ps) 3	Δ 6-elongase	278 As	SEQ ID NO: 7
D5-Des (Ps)	Δ 5-desaturase	498 As	SEQ ID NO: 9
D12-Des (Ps)	Δ 12-desaturase	398 As	SEQ ID NO: 25
O3-Des (Ps)	ω 3-desaturase	363 As	SEQ ID NO: 23

- 10 To prepare cDNA, total RNA of *Phytophthora sojae* was isolated with the aid of the RNAeasy kit from Qiagen (Valencia, CA, US). Poly-A⁺ RNA (mRNA) was isolated from the total RNA with the aid of oligo-dT cellulose (Sambrook et al., 1989). The RNA was subjected to reverse transcription using the Reverse
 15 Transcription System kit from Promega, and the synthesized cDNA was cloned into the lambda ZAP vector (lambda ZAP Gold, Stratagene). The cDNA was depackaged to give plasmid DNA, following the manufacturer's instructions. Then, the plasmid library was screened for the corresponding putative candidate
 20 genes (hybridization of bacterial clones, Sambrook et al. 1989), and positive clones were sequenced. The corresponding cDNA clones was then used for the PCR for cloning expression plasmids.
- 25 Example 4: Cloning of expression plasmids for the heterologous expression of *Phytophthora sojae* genes in yeasts

For the heterologous expression in yeasts, the corresponding sequences are amplified via PCR with corresponding specific primers and cloned into the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions.

- 5 Here, only those open reading frames of the genes are amplified which code for the PUFA proteins. In addition, a Kozak sequence (Cell 1986, 44:283-292) is attached at 5':

Gene	Base pairs	Primer	SEQ ID
D5-Des (Ps)	1497 bp	Fwd : gccatggcccccatcgagaccgac Rvs : ttagcccatgtggacggaca	SEQ ID NO: 27 SEQ ID NO: 28
D6-Des (Ps)	1371 bp	Fwd : accatggtggatggccccaagacca Rvs : ttacatggccgggaactcgagcagg	SEQ ID NO: 29 SEQ ID NO: 30
D12-Des (Ps)	1197 bp	Fwd : gccatggcgatcctgaaccgag Rvs : tagagcttgttctttaga	SEQ ID NO: 31 SEQ ID NO: 32
O3-Des (Ps)	1092 bp	Fwd: gccatggcgtccaagcaggagca Rvs: tcagttggccttagtcttggtcgcc	SEQ ID NO: 33 SEQ ID NO: 34
D6-Elo (Ps)	915 bp	Fwd: aagatggagacgaccttcgcgcgc Rvs: ttactgcgtcttcttggcgaccgcagcg	SEQ ID NO: 35 SEQ ID NO: 36
D6-Elo (Ps)_2	837 bp	Fwd: gccatggcgtcggagctgctgca Rvs: ttagaggttcttcttggccgg	SEQ ID NO: 37 SEQ ID NO: 38
D6-Elo (Ps)_3	837 bp	Fwd: accatgtcggccgacctgctgc Rvs: ttagagcttcttcttggc	SEQ ID NO: 39 SEQ ID NO: 40

Composition of the PCR mix (50 μ l):

- 5.00 μ l template cDNA
- 5 5.00 μ l 10x buffer (Advantage polymerase)+ 25 mM MgCl₂
- 5.00 μ l 2 mM dNTP
- 1.25 μ l of each primer (10 pmol/ μ l of the 5'-ATG and of the 3'-stop primer)
- 0.50 μ l Advantage polymerase
- 10 The Advantage polymerase from Clontech is employed.
- PCR reaction conditions:
- Annealing temperature: 1 min 55°C
- Denaturation temperature: 1 min 94°C
- Elongation temperature: 2 min 72°C
- 15 Number of cycles: 35

The PCR products are incubated with the plasmid pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. Then, the incubation reactions are transformed into E. coli DH5 α -

20 cells (Invitrogen), following the manufacturer's instructions. Positive clones are identified by PCR (see reaction above), and the plasmid DNA is isolated (Qiagen Dneasy). The plasmids formed are verified by sequencing and transformed by electroporation (1500 V) into the Saccharomyces strain INVSc1

25 (Invitrogen). As a control, pYES2.1 (blank vector) is transformed in parallel. The selection of the transformed yeast is carried out on Uracil drop out complete minimal medium (CMdum) agar plates with 2% glucose. After the selection, in each case three transformants are selected for

30 the further functional expression.

To express the genes from *Phytophthora sojae*, precultures of in each case 5 ml Uracil drop out CMdum liquid medium with 2% (w/v) raffinose are first inoculated with the selected

35 transformants and incubated for 2 days at 30°C, 200 rpm. Then, 5 ml CMdum liquid medium (without Uracil) with 2% raffinose and 300 μ M various fatty acids are inoculated with the precultures to an OD₆₀₀ of 0.05. Expression is induced by the

addition of 2% (w/v) galactose. The cultures are incubated for a further 96 h at 20°C.

5 Example 5: Cloning of expression plasmids for the seed-specific expression in plants

To transform plants, further transformation vectors based on pSUN-USP were generated. To this end, NotI cleavage sites were inserted at the 5' and 3' end of the coding sequences, using
10 the following primer pairs (see the following table).

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
15 5.00 µl 10x buffer (Advantage polymerase) + 25 mM MgCl₂
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

20

PCR reaction conditions:

Annealing temperature: 1 min 55°C

denaturing temperature: 1 min 94°C

25 Elongation temperature: 2 min 72°C

Number of cycles: 35

Gene	Base pairs	Primer	SEQ ID
D5-Des (Ps)	1497 bp	Fwd : gcggccgcgccatggcccccatcgag accgac	SEQ ID NO: 41
		Rvs : gcggccgccttagcccatgtggacgga ca	SEQ ID NO: 42
D6-Des (Ps)	1371 bp	Fwd : gcggccgcaccatggtggatggcccc aagacca	SEQ ID NO: 43

		Rvs : gcggccgcttacatggccgggaactc gagcagg	SEQ ID NO: 44
D12- Des (Ps)	1197 bp	Fwd : gcggccgcgccatggcgatcctgaac ccgg Rvs : gcggccgctagagcttggttcttgtag a	SEQ ID NO: 45 SEQ ID NO: 46
O3- Des (Ps)	1092 bp	Fwd: gcggccgcgccatggcgccaagcag gagca Rvs: gcggccgctcagttggccttagtctt ggtcgcc	SEQ ID NO: 47 SEQ ID NO: 48
D6- Elo (Ps)	915 bp	Fwd: gcggccgcaagatggagacgaccttc gcgcgc Rvs: gcggccgcttaactgcgtcttcttggc gaccgcagcg	SEQ ID NO: 49 SEQ ID NO: 50
D6- Elo (Ps)_2	837 bp	Fwd: gcggccgcgccatggcgtcggagctg ctgca Rvs: gcggccgcttagaggttcttcttggc cgg	SEQ ID NO: 51 SEQ ID NO: 52
D6- Elo (Ps)_3	837 bp	Fwd: gcggccgcaccatgtcggccgacctg ctgc Rvs: gcggccgcttagagcttcttcttggc	SEQ ID NO: 53 SEQ ID NO: 54

The PCR products are incubated with the restriction enzyme NotI for 4 hours at 37°C. The plant expression vector pSUN300-USP is incubated in the same manner. Thereafter, the PCR products and the vector, which is 7624 bp in size, are separated by agarose gel electrophoresis and the corresponding

DNA fragments are excised. The DNA is purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products are ligated. The Rapid Ligation Kit from Roche is used for this purpose.

5 The resulting plasmids are verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P., Svab, Z., Maliga, P., (1994). The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant
10 Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the *Ostreococcus* gene from the *A. tumefaciens* Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese,
15 P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-code for octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession
20 X56240), where part of the noncoding region of the USP gene is comprised in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction following standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer
25 (Stratagene). (Primer sequence:
5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC
GGATCTGCTGGCTATGAA-3') [SEQ ID NO: 55].

The PCR fragment was recut with EcoRI/SalI and inserted into
30 the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP, which can be employed for the transformation of plants by means of *Agrobacterium tumefaciens*.

35 Example 6: Expression of *Phytophthora sojae* genes in yeasts

Yeasts which had been transformed with the plasmid pYES2.1 or the plasmids pYES-d4Des(Ps), pYES-d5Des(Ps), pYES-d6Des(Ps),

pYES-d12Des(Ps), pYES-o3Des(Ps), pYES-d6Elo(Ps), pYES-d6Elo-2(Ps) and pYES-d6Elo-3(Ps) as described in Example 4 were analyzed as follows:

- 5 The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMES) were prepared by acid methanolysis. To this
10 end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMES were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of
15 100 mM NaHCO₃, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na₂SO₄, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame
20 ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).
- 25 The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, *Lipids*. 36(8):761-766; Sayanova et al., 2001, *Journal of Experimental Botany*. 52(360):1581-1585, Sperling et al., 2001, *Arch. Biochem. Biophys.* 388(2):293-298 and Michaelson et al.,
30 1998, *FEBS Letters*. 439(3):215-218.

Example 7: Functional characterization of *Phytophthora sojae* genes:

35

The activity and substrate specificity of the individual genes can be determined after expression and feeding of various fatty acids. The substrates fed are present in large amounts

in all transgenic yeasts, which demonstrate the uptake of these fatty acids into the yeasts. The transgenic yeasts reveal the synthesis of novel fatty acids, the products of the genes. This means that the genes from *Phytophthora sojae* can be expressed functionally.

The substrate specificity of desaturases and elongases can be determined after expression in yeast by feeding, using a variety of yeasts. Descriptions for the determination of the individual activities can be found in WO 93/11245 for ω 3-desaturases, WO 94/11516 for Δ 12-desaturases, WO 93/06712, US 5,614,393, US5614393, WO 96/21022, WO0021557 and WO 99/27111 for Δ 6-desaturases, Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566 for Δ 4-desaturases, Hong et al. 2002, Lipids 37,863-868 for Δ 5-desaturases, for elongases in Zank, T.K. et al. Plant Journal 31:255-268, 2002.

The activity of the individual desaturases and elongases is calculated from the conversion rate using the formula [substrate/(substrate+product)*100].

Example 8: Generation of transgenic plants

a) Generation of transgenic oilseed rape plants (modified method of Moloney et al., 1992, Plant Cell Reports, 8:238-242)

In order to generate transgenic oilseed rape plants, binary vectors such as the pSUN plasmids, generated as described in Example 5, with genes from *Phytophthora sojae* are transformed into *Agrobacterium tumefaciens* C58C1:pGV2260 (Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788). To transform oilseed rape plants (Var. Drakkar, NPZ Nordeutsche Pflanzenzucht, Hohenlieth, Germany), a 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog 1962 Physiol. Plant. 15, 473) supplemented with 3% sucrose (3MS medium) is used. Petioles or hypocotyls of freshly germinated sterile oilseed rape plants (in each case approx. 1 cm²) were incubated with a

1:50 agrobacterial dilution for 5-10 minutes in a Petri dish. This is followed by 3 days of coincubation in the dark at 25°C on 3MS medium supplemented with 0.8% Bacto agar. The cultures were then grown on for 3 days at 16 hours light/

5

8 hours dark, and then grown on in a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxim sodium), 50 mg/l kanamycin, 20 µM benzylaminopurine (BAP), now supplemented with 1.6 g/l of glucose. Growing shoots were transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

10

15 Regenerated shoots were obtained on 2MS medium supplemented with kanamycin and Claforan; after rooting, they were transferred to compost and, after growing on for two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, and mature seeds were harvested and analyzed by lipid analysis for expression of the desaturase and/or elongase genes as described, for example, by Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566.

20

b) Generation of transgenic linseed plants

25

Transgenic linseed plants can be generated for example by the method of Bell et al., 1999, In Vitro Cell. Dev. Biol.-Plant. 35(6):456-465 by means of particle bombardment. Agrobacteria-mediated transformations can be generated for example by the method of Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

30

Example 9: Lipid extraction from seeds:

35

The effect of the genetic modification in plants, fungi, algae, ciliates or on the production of a desired compound (such as a fatty acid) can be determined by growing the modified microorganisms or the modified plant under suitable

conditions (such as those described above) and analyzing the medium and/or the cellular components for the elevated production of desired product (i.e. of the lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned processes, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940 and Browse et al. (1986) Analytic Biochemistry 152:141-145. The qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for the production of the desired compound, such as intermediates and by-products, in order to determine the overall production efficiency of the compound. The analytical methods comprise measuring the amount of nutrients in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measuring the biomass composition and the growth, analyzing the production of conventional metabolites of biosynthetic pathways and measuring gases which are generated during the fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, Ed., IRL Press, p. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl ester; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

The unambiguous detection for the presence of fatty acid products can be obtained by analyzing recombinant organisms using analytical standard methods: GC, GC-MS or TLC, as described on several occasions by Christie and the references therein (1997, in: Advances on Lipid Methodology, Fourth Edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [Gas chromatography/mass spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, grinding in a glass mill, liquid nitrogen and grinding or via other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction for one hour at 90°C in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane,

which leads to hydrolyzed oil and lipid compounds, which give transmethyated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to a GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 μ m, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and 5 minutes at 240°C. The identity of the resulting fatty acid methyl esters must be defined using standards which are available from commercial sources (i.e. Sigma).

10

Plant material is initially homogenized mechanically by comminuting in a pestle and mortar to make it more amenable to extraction.

15 This is followed by heating at 100°C for 10 minutes and, after cooling on ice, by resedimentation. The cell sediment is hydrolyzed for one hour at 90°C with 1 M methanolic sulfuric acid and 2% dimethoxypropane, and the lipids are transmethyated. The resulting fatty acid methyl esters
20 (FAMES) are extracted in petroleum ether. The extracted FAMES are analyzed by gas liquid chromatography using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170°C to 240°C in 20 minutes and 5 minutes at 240°C. The identity of the fatty acid
25 methyl esters is confirmed by comparison with corresponding FAME standards (Sigma). The identity and position of the double bond can be analyzed further by suitable chemical derivatization of the FAME mixtures, for example to give 4,4-dimethoxyoxazoline derivatives (Christie, 1998) by means of
30 GC-MS.

Equivalents:

Many equivalents of the specific embodiments according to the invention described herein can be identified or found by the
35 skilled worker resorting simply to routine experiments. These equivalents are intended to be within the scope of the patent claims.

SEQUENCE LISTING

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<140> PF 56465

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Val Pro Phe Leu Ile Asn Thr Val Tyr Arg Arg Gly Val Tyr His Ser	115	120	125	
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tac tac ttc gtg agc gcg cac acg cgg gac att tgg tgg aag cgc tac Tyr Tyr Phe Val Ser Ala His Thr Arg Asp Ile Trp Trp Lys Arg Tyr 195 200 205			624
ctg acg ctc att cag ttg gtg cag ttc gtg acc atg aac gtg cag ggc Leu Thr Leu Ile Gln Leu Val Gln Phe Val Thr Met Asn Val Gln Gly 210 215 220			672
tac ctc atg tac tcg cgc cag tgc cca ggc atg ccg ccc aag atc ccg Tyr Leu Met Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Ile Pro 225 230 235 240			720
ctc atc tat ctg gcc tac gtg cag tcg ctc ttc tgg ctg ttc gtc aac Leu Ile Tyr Leu Ala Tyr Val Gln Ser Leu Phe Trp Leu Phe Val Asn 245 250 255			768
ttc tac gtg cgc tcg tac gtg ctc gcc ccc aag aag acc aag gcg tcc Phe Tyr Val Arg Ser Tyr Val Leu Ala Pro Lys Lys Thr Lys Ala Ser 260 265 270			816
ccg gcc aag aag aac ctc taa Pro Ala Lys Lys Asn Leu			837

275

<210> 6

<211> 278

<212> PRT

<213> Phytophthora sojae

<400> 6

Met Ala Ser Glu Leu Leu Gln Ser Tyr Tyr Glu Trp Ala Asn Ala Thr
 1 5 10 15

Glu Ile Lys Val Leu Asp Trp Val Asp Pro Glu Gly Gly Trp Lys Val
 20 25 30

His Pro Met Ala Asp Tyr Pro Leu Ala Asn Phe Ala Ser Val Phe Ala
 35 40 45

Ile Cys Val Gly Tyr Leu Leu Phe Val Ile Phe Gly Thr Ala Leu Met
 50 55 60

Lys Met Gly Ile Pro Ala Ile Lys Thr Ser Pro Ile Gln Phe Ile Tyr
 65 70 75 80

Asn Pro Ile Gln Val Ile Ala Cys Ser Tyr Met Phe Val Glu Thr Ala
 85 90 95

Ile Gln Ala Tyr Arg Asn Gly Tyr Ser Pro Ala Pro Cys Asn Ala Phe
 100 105 110

Lys Thr Asp Ala Pro Val Met Gly Asn Val Leu Tyr Leu Phe Tyr Leu
 115 120 125

Ser Lys Met Leu Asp Leu Cys Asp Thr Phe Phe Ile Val Val Gly Lys
 130 135 140

Lys Trp Arg Gln Leu Ser Phe Leu His Val Tyr His His Leu Ser Val
 145 150 155 160

Leu Leu Met Tyr Tyr Ile Val Phe Arg Val Ala Gln Asp Gly Asp Ser
 165 170 175

Tyr Ala Ser Val Val Leu Asn Gly Phe Val His Thr Ile Met Tyr Thr
 180 185 190

Tyr Tyr Phe Val Ser Ala His Thr Arg Asp Ile Trp Trp Lys Arg Tyr

195 200 205
 Leu Thr Leu Ile Gln Leu Val Gln Phe Val Thr Met Asn Val Gln Gly
 210 215 220
 Tyr Leu Met Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Ile Pro
 225 230 235 240
 Leu Ile Tyr Leu Ala Tyr Val Gln Ser Leu Phe Trp Leu Phe Val Asn
 245 250 255
 Phe Tyr Val Arg Ser Tyr Val Leu Ala Pro Lys Lys Thr Lys Ala Ser
 260 265 270
 Pro Ala Lys Lys Asn Leu
 275

<210> 7

<211> 837

<212> DNA

<213> Phytophthora sojae

<220>

<221> CDS

<222> (1)..(837)

<223> Delta-6 elongase

<400> 7

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 Met Ser Ala Asp Leu Leu Gln Ser Tyr Tyr Asp Trp Thr Asn Ala Thr
 1 5 10 15

gag gcc aag ctg ctc gac tgg gtg gac ccc gaa ggc ggc tgg aag gtc 96
 Glu Ala Lys Leu Leu Asp Trp Val Asp Pro Glu Gly Gly Trp Lys Val
 20 25 30

cac ccc atg gcc gac tac ccg ctc gcc aac ttc gcc agc gtc tac gcc 144
 His Pro Met Ala Asp Tyr Pro Leu Ala Asn Phe Ala Ser Val Tyr Ala
 35 40 45

atc tgc gtc ggc tac ctg ctc ttc gtc atc ttc ggc acg gcc ctg atg 192
 Ile Cys Val Gly Tyr Leu Leu Phe Val Ile Phe Gly Thr Ala Leu Met
 50 55 60

aag atg ggc atc ccc gcc atc aag acg agc ccc atc cag ttc atc tac 240
 Lys Met Gly Ile Pro Ala Ile Lys Thr Ser Pro Ile Gln Phe Ile Tyr
 65 70 75 80

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aac ccc atc cag gtc atc gcc tgc tcc tac atg tgc gtg gag gcc gcc      288
Asn Pro Ile Gln Val Ile Ala Cys Ser Tyr Met Cys Val Glu Ala Ala
      85                      90                      95

atc cag gcc tac cgc aac ggc tac agc gcg gcg ccc tgc aac gcc ttc      336
Ile Gln Ala Tyr Arg Asn Gly Tyr Ser Ala Ala Pro Cys Asn Ala Phe
      100                    105                    110

aag gcg gac gcg ccc gtc atg ggc aac gtg ctc tac ctg ttc tac ctg      384
Lys Ala Asp Ala Pro Val Met Gly Asn Val Leu Tyr Leu Phe Tyr Leu
      115                    120                    125

tcc aag atg ctg gac ctg tgc gac acg gtc ttc atc atc ctc ggc aag      432
Ser Lys Met Leu Asp Leu Cys Asp Thr Val Phe Ile Ile Leu Gly Lys
      130                    135                    140

aag tgg aag cag ctc tcc atc ctg cac gtg tac cac cac ctg acc gtg      480
Lys Trp Lys Gln Leu Ser Ile Leu His Val Tyr His His Leu Thr Val
      145                    150                    155                    160

ctc ttc gtc tac tac gtg acg ttc cgc gcc gcc cag gac ggc gac tcg      528
Leu Phe Val Tyr Tyr Val Thr Phe Arg Ala Ala Gln Asp Gly Asp Ser
      165                    170                    175

tac gcc acc atc gtg ctc aac ggc ttc gtg cac acc atc atg tac acg      576
Tyr Ala Thr Ile Val Leu Asn Gly Phe Val His Thr Ile Met Tyr Thr
      180                    185                    190

tac tac ttc gtg agc gcg cac acg cgc aac att tgg tgg aag aag tac      624
Tyr Tyr Phe Val Ser Ala His Thr Arg Asn Ile Trp Trp Lys Lys Tyr
      195                    200                    205

ctc acg cgc atc cag ctc atc cag ttc gtg acc atg aac gtg cag ggc      672
Leu Thr Arg Ile Gln Leu Ile Gln Phe Val Thr Met Asn Val Gln Gly
      210                    215                    220

tac ctg acg tac tcg cgc cag tgc ccg ggc atg ccc ccc aag gtg ccg      720
Tyr Leu Thr Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Val Pro
      225                    230                    235                    240

ctc atg tac ctc gtg tac gtg cag tcg ctc ttc tgg ctc ttc atg aac      768
Leu Met Tyr Leu Val Tyr Val Gln Ser Leu Phe Trp Leu Phe Met Asn
      245                    250                    255

ttc tac atc cgc gcg tac gtc ttc ggc ccc aag aag ccc gcc gtc gag      816
Phe Tyr Ile Arg Ala Tyr Val Phe Gly Pro Lys Lys Pro Ala Val Glu
      260                    265                    270

gac gcc aag aag aag ctc taa      837
Asp Ala Lys Lys Lys Leu
      275

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<210> 8

<211> 278

<212> PRT

<213> Phytophthora sojae

<400> 8

Met Ser Ala Asp Leu Leu Gln Ser Tyr Tyr Asp Trp Thr Asn Ala Thr
1 5 10 15

Glu Ala Lys Leu Leu Asp Trp Val Asp Pro Glu Gly Gly Trp Lys Val
20 25 30

His Pro Met Ala Asp Tyr Pro Leu Ala Asn Phe Ala Ser Val Tyr Ala
35 40 45

Ile Cys Val Gly Tyr Leu Leu Phe Val Ile Phe Gly Thr Ala Leu Met
50 55 60

Lys Met Gly Ile Pro Ala Ile Lys Thr Ser Pro Ile Gln Phe Ile Tyr
65 70 75 80

Asn Pro Ile Gln Val Ile Ala Cys Ser Tyr Met Cys Val Glu Ala Ala
85 90 95

Ile Gln Ala Tyr Arg Asn Gly Tyr Ser Ala Ala Pro Cys Asn Ala Phe
100 105 110

Lys Ala Asp Ala Pro Val Met Gly Asn Val Leu Tyr Leu Phe Tyr Leu
115 120 125

Ser Lys Met Leu Asp Leu Cys Asp Thr Val Phe Ile Ile Leu Gly Lys
130 135 140

Lys Trp Lys Gln Leu Ser Ile Leu His Val Tyr His His Leu Thr Val
145 150 155 160

Leu Phe Val Tyr Tyr Val Thr Phe Arg Ala Ala Gln Asp Gly Asp Ser
165 170 175

Tyr Ala Thr Ile Val Leu Asn Gly Phe Val His Thr Ile Met Tyr Thr
180 185 190

Tyr Tyr Phe Val Ser Ala His Thr Arg Asn Ile Trp Trp Lys Lys Tyr
195 200 205

Leu Thr Arg Ile Gln Leu Ile Gln Phe Val Thr Met Asn Val Gln Gly
210 215 220

Tyr Leu Thr Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Val Pro
225 230 235 240

Leu Met Tyr Leu Val Tyr Val Gln Ser Leu Phe Trp Leu Phe Met Asn
245 250 255

Phe Tyr Ile Arg Ala Tyr Val Phe Gly Pro Lys Lys Pro Ala Val Glu
 260 265 270

Asp Ala Lys Lys Lys Leu
 275

<210> 9

<211> 1497

<212> DNA

<213> *Phytophthora sojae*

<220>

<221> CDS

<222> (1)..(1497)

<223> Delta-5 desaturase

<400> 9

atg gcc ccc atc gag acc gac aag gcc gtg agt gcc aac gag ggc ctg 48
 Met Ala Pro Ile Glu Thr Asp Lys Ala Val Ser Ala Asn Glu Gly Leu
 1 5 10 15

cac cag cgc aag ggc gcc gcc tcg gcc gac aag gac gcc acc tac acg 96
 His Gln Arg Lys Gly Ala Ala Ser Ala Asp Lys Asp Ala Thr Tyr Thr
 20 25 30

tgg cag gac gtg gcc aag cac aac acg gcc aag agc gcc tgg gtc atc 144
 Trp Gln Asp Val Ala Lys His Asn Thr Ala Lys Ser Ala Trp Val Ile
 35 40 45

atc cgc ggc gtc gtc tac gac gtc act gat act ctg aaa aca ccc caa 192
 Ile Arg Gly Val Val Tyr Asp Val Thr Asp Thr Leu Lys Thr Pro Gln
 50 55 60

agt aaa atc cta acc tgt tcc tgc gtg atg gca cca cca gaa tgg gcg 240
 Ser Lys Ile Leu Thr Cys Ser Cys Val Met Ala Pro Pro Glu Trp Ala
 65 70 75 80

gac cgt cac cct ggc ggc cgc gag ctc gtg ctg ctg cac gcc gga cgc 288
 Asp Arg His Pro Gly Gly Arg Glu Leu Val Leu Leu His Ala Gly Arg
 85 90 95

gag tgc acg gac acg ttc gac tcg tac cac ccc ttc tcc aac cgc gcc 336
 Glu Cys Thr Asp Thr Phe Asp Ser Tyr His Pro Phe Ser Asn Arg Ala
 100 105 110

gac aag atc ctg gcc aag tac gcc gtt ggt aag ctc gtg ggc ggc tcc 384
 Asp Lys Ile Leu Ala Lys Tyr Ala Val Gly Lys Leu Val Gly Gly Ser
 115 120 125

gag ttc ccc cag tac aag ccc gac acg ggc ttc tac aag gag tgc tgc 432

Glu	Phe	Pro	Gln	Tyr	Lys	Pro	Asp	Thr	Gly	Phe	Tyr	Lys	Glu	Cys	Cys	
130						135					140					
gag	cgc	gtg	cac	cag	tac	ttt	aag	gac	aac	aac	ctg	gac	ccg	cgc	agc	480
Glu	Arg	Val	His	Gln	Tyr	Phe	Lys	Asp	Asn	Asn	Leu	Asp	Pro	Arg	Ser	
145				150					155						160	
ccg	tac	tcg	ggc	atg	tgg	cgc	atg	atg	atc	gtc	gct	gcg	ctc	ggc	gcc	528
Pro	Tyr	Ser	Gly	Met	Trp	Arg	Met	Met	Ile	Val	Ala	Ala	Leu	Gly	Ala	
			165						170					175		
atc	tcg	tac	ctg	ggc	atg	aac	cag	ctg	ctc	tcg	gac	aac	atc	tac	gcg	576
Ile	Ser	Tyr	Leu	Gly	Met	Asn	Gln	Leu	Leu	Ser	Asp	Asn	Ile	Tyr	Ala	
			180					185					190			
cac	tac	gcg	tgg	ggc	gcc	ctc	ttc	ggc	gtg	tgc	cag	gcg	ctg	ccg	ctg	624
His	Tyr	Ala	Trp	Gly	Ala	Leu	Phe	Gly	Val	Cys	Gln	Ala	Leu	Pro	Leu	
		195					200					205				
ctc	cac	gtg	atg	cac	gac	gcg	tcg	cac	gcc	gcc	atc	acc	agc	agc	ccc	672
Leu	His	Val	Met	His	Asp	Ala	Ser	His	Ala	Ala	Ile	Thr	Ser	Ser	Pro	
	210					215					220					
acg	ggc	tgg	agg	ctc	att	ggc	cgc	ttc	gcg	atg	gac	tgg	gtg	gcc	ggc	720
Thr	Gly	Trp	Arg	Leu	Ile	Gly	Arg	Phe	Ala	Met	Asp	Trp	Val	Ala	Gly	
225				230					235					240		
gcc	aac	atg	gta	tcg	tgg	ctc	aac	cag	cac	gtt	gtg	ggc	cac	cac	atc	768
Ala	Asn	Met	Val	Ser	Trp	Leu	Asn	Gln	His	Val	Val	Gly	His	His	Ile	
			245					250					255			
tac	aca	aac	gtg	gcc	ggc	gct	gac	ccc	gac	ctt	ccc	gtg	gac	ttc	aag	816
Tyr	Thr	Asn	Val	Ala	Gly	Ala	Asp	Pro	Asp	Leu	Pro	Val	Asp	Phe	Lys	
			260					265					270			
agc	gac	gtg	cgc	cgc	att	gtg	tac	cgt	cag	gtg	ctg	ctg	ccc	atc	tac	864
Ser	Asp	Val	Arg	Arg	Ile	Val	Tyr	Arg	Gln	Val	Leu	Leu	Pro	Ile	Tyr	
		275					280					285				
aag	ttc	cag	cac	ttg	tac	ctg	ccg	ccg	ctg	tac	ggc	gtg	ctc	ggc	ctc	912
Lys	Phe	Gln	His	Leu	Tyr	Leu	Pro	Pro	Leu	Tyr	Gly	Val	Leu	Gly	Leu	
	290					295					300					
aag	ttc	cgc	gtg	cag	gac	att	ttc	gag	acg	ttc	atc	tcg	ctc	acg	aac	960
Lys	Phe	Arg	Val	Gln	Asp	Ile	Phe	Glu	Thr	Phe	Ile	Ser	Leu	Thr	Asn	
305				310					315					320		
ggt	ccg	ctg	cgc	gtg	aac	ccg	cac	tca	gtc	ggc	gac	tgg	gtc	gag	atg	1008
Gly	Pro	Leu	Arg	Val	Asn	Pro	His	Ser	Val	Gly	Asp	Trp	Val	Glu	Met	
			325						330					335		
atc	ctg	tcc	aag	gcc	ttc	tgg	gcg	ttc	tac	cgc	atc	tac	atc	ccg	ctg	1056
Ile	Leu	Ser	Lys	Ala	Phe	Trp	Ala	Phe	Tyr	Arg	Ile	Tyr	Ile	Pro	Leu	
			340					345					350			
gtc	gtg	ctg	cag	gtg	gac	tcg	tcc	cgc	ttc	tgg	ggc	gtc	ttc	ttc	ctg	1104
Val	Val	Leu	Gln	Val	Asp	Ser	Ser	Arg	Phe	Trp	Gly	Val	Phe	Phe	Leu	
		355					360					365				
gcc	gag	ttc	atg	acg	ggc	tgg	tac	ctg	gcc	ttc	aac	ttc	cag	gtg	agc	1152
Ala	Glu	Phe	Met	Thr	Gly	Trp	Tyr	Leu	Ala	Phe	Asn	Phe	Gln	Val	Ser	
	370					375					380					

cac gtc tcc acg gcc tgc gaa tac ccg ggc ggt gac gag gag gtg acg 1200
 His Val Ser Thr Ala Cys Glu Tyr Pro Gly Gly Asp Glu Glu Val Thr
 385 390 395 400

gcc atc gag gac gag tgg gct gtc tcg cag atc aag tcg tcg gtg gac 1248
 Ala Ile Glu Asp Glu Trp Ala Val Ser Gln Ile Lys Ser Ser Val Asp
 405 410 415

tac ggc cac ggc tcg ttc ctc acg gcg ttc ctc acg ggc gcg ctg aac 1296
 Tyr Gly His Gly Ser Phe Leu Thr Ala Phe Leu Thr Gly Ala Leu Asn
 420 425 430

tac cag gtg acc cac cac ctc ttc ccg ggc gtc tcg cag tac cac tac 1344
 Tyr Gln Val Thr His His Leu Phe Pro Gly Val Ser Gln Tyr His Tyr
 435 440 445

ccg gcc atc gcg ccg atc atc atc gac gtg tgc aac aaa tac aag atc 1392
 Pro Ala Ile Ala Pro Ile Ile Ile Asp Val Cys Asn Lys Tyr Lys Ile
 450 455 460

aag tac acg gtg ctc ccc acg ttc acg gag gcg ctg gcc ggc cac ttc 1440
 Lys Tyr Thr Val Leu Pro Thr Phe Thr Glu Ala Leu Ala Gly His Phe
 465 470 475 480

gac cac ctc gtc gtc atg ggc aag atg ggc aag ccc gtg tcc gtc cac 1488
 Asp His Leu Val Val Met Gly Lys Met Gly Lys Pro Val Ser Val His
 485 490 495

atg ggc taa 1497
 Met Gly

<210> 10

<211> 498

<212> PRT

<213> Phytophthora sojae

<400> 10

Met Ala Pro Ile Glu Thr Asp Lys Ala Val Ser Ala Asn Glu Gly Leu
 1 5 10 15

His Gln Arg Lys Gly Ala Ala Ser Ala Asp Lys Asp Ala Thr Tyr Thr
 20 25 30

Trp Gln Asp Val Ala Lys His Asn Thr Ala Lys Ser Ala Trp Val Ile
 35 40 45

Ile Arg Gly Val Val Tyr Asp Val Thr Asp Thr Leu Lys Thr Pro Gln
 50 55 60

Ser Lys Ile Leu Thr Cys Ser Cys Val Met Ala Pro Pro Glu Trp Ala

65					70						75				80
Asp	Arg	His	Pro	Gly	Gly	Arg	Glu	Leu	Val	Leu	Leu	His	Ala	Gly	Arg
				85					90					95	
Glu	Cys	Thr	Asp	Thr	Phe	Asp	Ser	Tyr	His	Pro	Phe	Ser	Asn	Arg	Ala
			100					105					110		
Asp	Lys	Ile	Leu	Ala	Lys	Tyr	Ala	Val	Gly	Lys	Leu	Val	Gly	Gly	Ser
		115					120					125			
Glu	Phe	Pro	Gln	Tyr	Lys	Pro	Asp	Thr	Gly	Phe	Tyr	Lys	Glu	Cys	Cys
	130						135				140				
Glu	Arg	Val	His	Gln	Tyr	Phe	Lys	Asp	Asn	Asn	Leu	Asp	Pro	Arg	Ser
145					150					155					160
Pro	Tyr	Ser	Gly	Met	Trp	Arg	Met	Met	Ile	Val	Ala	Ala	Leu	Gly	Ala
				165					170					175	
Ile	Ser	Tyr	Leu	Gly	Met	Asn	Gln	Leu	Leu	Ser	Asp	Asn	Ile	Tyr	Ala
			180					185					190		
His	Tyr	Ala	Trp	Gly	Ala	Leu	Phe	Gly	Val	Cys	Gln	Ala	Leu	Pro	Leu
		195					200					205			
Leu	His	Val	Met	His	Asp	Ala	Ser	His	Ala	Ala	Ile	Thr	Ser	Ser	Pro
	210					215					220				
Thr	Gly	Trp	Arg	Leu	Ile	Gly	Arg	Phe	Ala	Met	Asp	Trp	Val	Ala	Gly
225					230					235					240
Ala	Asn	Met	Val	Ser	Trp	Leu	Asn	Gln	His	Val	Val	Gly	His	His	Ile
				245					250					255	
Tyr	Thr	Asn	Val	Ala	Gly	Ala	Asp	Pro	Asp	Leu	Pro	Val	Asp	Phe	Lys
			260					265					270		
Ser	Asp	Val	Arg	Arg	Ile	Val	Tyr	Arg	Gln	Val	Leu	Leu	Pro	Ile	Tyr
		275					280					285			
Lys	Phe	Gln	His	Leu	Tyr	Leu	Pro	Pro	Leu	Tyr	Gly	Val	Leu	Gly	Leu
	290					295					300				
Lys	Phe	Arg	Val	Gln	Asp	Ile	Phe	Glu	Thr	Phe	Ile	Ser	Leu	Thr	Asn
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<221> CDS

<222> (1)..(903)

<223> Delta-5 elongase

<400> 11

atg	tct	gct	tct	gga	gct	ttg	ttg	cct	gct	att	gct	ttc	gct	gct	tac	48
Met	Ser	Ala	Ser	Gly	Ala	Leu	Leu	Pro	Ala	Ile	Ala	Phe	Ala	Ala	Tyr	
1				5					10					15		

gct	tac	gct	acc	tac	gct	tat	gct	ttc	gag	tgg	tct	cat	gct	aac	gga	96
Ala	Tyr	Ala	Thr	Tyr	Ala	Tyr	Ala	Phe	Glu	Trp	Ser	His	Ala	Asn	Gly	
			20					25					30			

atc	gat	aac	gtg	gat	gct	aga	gag	tgg	att	gga	gct	ttg	tct	ttg	aga	144
Ile	Asp	Asn	Val	Asp	Ala	Arg	Glu	Trp	Ile	Gly	Ala	Leu	Ser	Leu	Arg	
		35					40					45				

ctc	cct	gca	att	gct	acc	acc	atg	tac	ctc	ttg	ttc	tgc	ctt	gtg	gga	192
Leu	Pro	Ala	Ile	Ala	Thr	Thr	Met	Tyr	Leu	Leu	Phe	Cys	Leu	Val	Gly	
	50					55					60					

cct	aga	ttg	atg	gct	aag	agg	gag	gct	ttt	gat	cct	aag	gga	ttc	atg	240
Pro	Arg	Leu	Met	Ala	Lys	Arg	Glu	Ala	Phe	Asp	Pro	Lys	Gly	Phe	Met	
65					70				75					80		

ctc	gct	tac	aac	gct	tac	caa	acc	gct	ttc	aac	gtt	gtg	gtg	ctc	gga	288
Leu	Ala	Tyr	Asn	Ala	Tyr	Gln	Thr	Ala	Phe	Asn	Val	Val	Val	Leu	Gly	
			85					90						95		

atg	ttc	gct	aga	gag	atc	tct	gga	ttg	gga	caa	cct	gtt	tgg	gga	tct	336
Met	Phe	Ala	Arg	Glu	Ile	Ser	Gly	Leu	Gly	Gln	Pro	Val	Trp	Gly	Ser	
			100				105						110			

act	atg	cct	tgg	agc	gat	agg	aag	tcc	ttc	aag	att	ttg	ttg	gga	gtg	384
Thr	Met	Pro	Trp	Ser	Asp	Arg	Lys	Ser	Phe	Lys	Ile	Leu	Leu	Gly	Val	
		115				120						125				

tgg	ctc	cat	tac	aac	aat	aag	tac	ctc	gag	ttg	ttg	gat	act	gtg	ttc	432
Trp	Leu	His	Tyr	Asn	Asn	Lys	Tyr	Leu	Glu	Leu	Leu	Asp	Thr	Val	Phe	
	130				135						140					

atg	gtg	gct	agg	aaa	aag	acc	aag	cag	ctc	tct	ttc	ttg	cat	gtg	tac	480
Met	Val	Ala	Arg	Lys	Lys	Thr	Lys	Gln	Leu	Ser	Phe	Leu	His	Val	Tyr	
145				150				155						160		

cat	cat	gct	ttg	ttg	att	tgg	gct	tgg	tgg	ctt	gtt	tgt	cat	ctc	atg	528
His	His	Ala	Leu	Leu	Ile	Trp	Ala	Trp	Trp	Leu	Val	Cys	His	Leu	Met	
			165					170						175		

gct	acc	aac	gat	tgc	atc	gat	gct	tat	ttc	gga	gct	gct	tgc	aac	tct	576
Ala	Thr	Asn	Asp	Cys	Ile	Asp	Ala	Tyr	Phe	Gly	Ala	Ala	Cys	Asn	Ser	
		180					185					190				

ttc	atc	cac	atc	gtg	atg	tac	tcc	tac	tac	ctc	atg	tct	gct	ttg	gga	624
Phe	Ile	His	Ile	Val	Met	Tyr	Ser	Tyr	Tyr	Leu	Met	Ser	Ala	Leu	Gly	
		195				200						205				

att	aga	tgc	cct	tgg	aag	aga	tat	atc	acc	cag	gct	cag	atg	ttg	caa	672
Ile	Arg	Cys	Pro	Trp	Lys	Arg	Tyr	Ile	Thr	Gln	Ala	Gln	Met	Leu	Gln	
	210				215					220						

ttc	gtg	atc	gtg	ttc	gct	cat	gct	gtt	ttc	gtg	ctc	aga	caa	aag	cac	720
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Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
 225 230 235 240
 tgc cct gtt act ttg cct tgg gca caa atg ttc gtg atg aca aat atg 768
 Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
 245 250 255
 ttg gtg ctc ttc gga aac ttc tac ctc aag gct tac tct aac aag tct 816
 Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270
 agg gga gat gga gct tct tct gtt aag cct gct gag act act aga gca 864
 Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
 275 280 285
 cct tct gtg aga aga acc agg tcc agg aag atc gat tga 903
 Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<210> 12

<211> 300

<212> PRT

<213> *Ostreococcus tauri*

<400> 12

Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
 1 5 10 15
 Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
 20 25 30
 Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45
 Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60
 Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80
 Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
 100 105 110
 Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
 115 120 125

Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
 130 135 140

Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
 145 150 155 160

His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
 165 170 175

Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
 180 185 190

Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
 195 200 205

Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
 210 215 220

Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
 225 230 235 240

Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
 245 250 255

Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270

Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
 275 280 285

Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<210> 13

<211> 903

<212> DNA

<213> *Ostreococcus tauri*

<220>

<221> CDS

<222> (1)..(903)

<223> Delta-5 elongase

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<400> 13
atg agc gcc tcc ggt gcg ctg ctg ccc gcg atc gcg ttc gcc gcg tac      48
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1          5          10          15

gcg tac gcg acg tac gcc tac gcc ttt gag tgg tcg cac gcg aat ggc      96
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
          20          25          30

atc gac aac gtc gac gcg cgc gag tgg atc ggt gcg ctg tcg ttg agg      144
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
          35          40          45

ctc ccg gcg atc gcg acg acg atg tac ctg ttg ttc tgc ctg gtc gga      192
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
          50          55          60

ccg agg ttg atg gcg aag cgc gag gcg ttc gac ccg aag ggg ttc atg      240
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65          70          75          80

ctg gcg tac aat gcg tat cag acg gcg ttc aac gtc gtc gtg ctc ggg      288
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
          85          90          95

atg ttc gcg cga gag atc tcg ggg ctg ggg cag ccc gtg tgg ggg tca      336
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
          100          105          110

acc atg ccg tgg agc gat aga aaa tcg ttt aag atc ctc ctc ggg gtg      384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
          115          120          125

tgg ttg cac tac aac aac aaa tat ttg gag cta ttg gac act gtg ttc      432
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
          130          135          140

atg gtt gcg cgc aag aag acg aag cag ttg agc ttc ttg cac gtt tat      480
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
          145          150          155          160

cat cac gcc ctg ttg atc tgg gcg tgg tgg ttg gtg tgt cac ttg atg      528
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
          165          170          175

gcc acg aac gat tgt atc gat gcc tac ttc ggc gcg gcg tgc aac tcg      576
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
          180          185          190

ttc att cac atc gtg atg tac tcg tat tat ctc atg tcg gcg ctc ggc      624
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
          195          200          205

att cga tgc ccg tgg aag cga tac atc acc cag gct caa atg ctc caa      672
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
          210          215          220

ttc gtc att gtc ttc gcg cac gcc gtg ttc gtg ctg cgt cag aag cac      720
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
          225          230          235          240

tgc ccg gtc acc ctt cct tgg gcg caa atg ttc gtc atg acg aac atg      768
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
          245          250          255

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ctc gtg ctc ttc ggg aac ttc tac ctc aag gcg tac tcg aac aag tcg 816
 Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270

cgc ggc gac ggc gcg agt tcc gtg aaa cca gcc gag acc acg cgc gcg 864
 Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
 275 280 285

ccc agc gtg cga cgc acg cga tct cga aaa att gac taa 903
 Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<210> 14

<211> 300

<212> PRT

<213> *Ostreococcus tauri*

<400> 14

Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
 1 5 10 15

Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
 20 25 30

Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45

Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60

Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80

Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95

Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
 100 105 110

Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
 115 120 125

Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
 130 135 140

Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
 145 150 155 160

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His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
 165 170 175

Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
 180 185 190

Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
 195 200 205

Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
 210 215 220

Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
 225 230 235 240

Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
 245 250 255

Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270

Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
 275 280 285

Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<210> 15

<211> 1512

<212> DNA

<213> *Thalassiosira pseudonana*

<220>

<221> CDS

<222> (1)..(1512)

<223> Delta-4 desaturase

<400> 15

atg tgc aac ggc aac ctc cca gca tcc acc gca cag ctc aag tcc acc 48
 Met Cys Asn Gly Asn Leu Pro Ala Ser Thr Ala Gln Leu Lys Ser Thr
 1 5 10 15

tcg aag ccc cag cag caa cat gag cat cgc acc atc tcc aag tcc gag 96

Ser	Lys	Pro	Gln	Gln	Gln	His	Glu	His	Arg	Thr	Ile	Ser	Lys	Ser	Glu	
			20					25					30			
ctc	gcc	caa	cac	aac	acg	ccc	aaa	tca	gca	tgg	tgt	gcc	gtc	cac	tcc	144
Leu	Ala	Gln	His	Asn	Thr	Pro	Lys	Ser	Ala	Trp	Cys	Ala	Val	His	Ser	
		35					40					45				
act	ccc	gcc	acc	gac	cca	tcc	cac	tcc	aac	aac	aaa	caa	cac	gca	cac	192
Thr	Pro	Ala	Thr	Asp	Pro	Ser	His	Ser	Asn	Asn	Lys	Gln	His	Ala	His	
	50					55					60					
cta	gtc	ctc	gac	att	acc	gac	ttt	gcg	tcc	cgc	cat	cca	ggg	gga	gac	240
Leu	Val	Leu	Asp	Ile	Thr	Asp	Phe	Ala	Ser	Arg	His	Pro	Gly	Gly	Asp	
65					70					75					80	
ctc	atc	ctc	ctc	gct	tcc	ggc	aaa	gac	gcc	tcg	gtg	ctg	ttt	gaa	aca	288
Leu	Ile	Leu	Leu	Ala	Ser	Gly	Lys	Asp	Ala	Ser	Val	Leu	Phe	Glu	Thr	
				85					90					95		
tac	cat	cca	cgt	gga	gtt	ccg	acg	tct	ctc	att	caa	aag	ctg	cag	att	336
Tyr	His	Pro	Arg	Gly	Val	Pro	Thr	Ser	Leu	Ile	Gln	Lys	Leu	Gln	Ile	
			100					105					110			
gga	gtg	atg	gag	gag	gag	gcg	ttt	cgg	gat	tcg	ttt	tac	agt	tgg	act	384
Gly	Val	Met	Glu	Glu	Glu	Ala	Phe	Arg	Asp	Ser	Phe	Tyr	Ser	Trp	Thr	
		115					120					125				
gat	tct	gac	ttt	tat	act	gtg	ttg	aag	agg	agg	gtt	gtg	gag	cgg	ttg	432
Asp	Ser	Asp	Phe	Tyr	Thr	Val	Leu	Lys	Arg	Arg	Val	Val	Glu	Arg	Leu	
	130					135					140					
gag	gag	agg	ggg	ttg	gac	agg	agg	gga	tcg	aaa	gag	att	tgg	atc	aag	480
Glu	Glu	Arg	Gly	Leu	Asp	Arg	Arg	Gly	Ser	Lys	Glu	Ile	Trp	Ile	Lys	
145					150					155					160	
gct	ttg	ttc	ttg	ttg	gtt	gga	ttt	tgg	tac	tgt	ttg	tac	aag	atg	tat	528
Ala	Leu	Phe	Leu	Leu	Val	Gly	Phe	Trp	Tyr	Cys	Leu	Tyr	Lys	Met	Tyr	
				165					170					175		
act	acg	tcg	gat	atc	gat	cag	tac	ggg	att	gcc	att	gcc	tat	tct	att	576
Thr	Thr	Ser	Asp	Ile	Asp	Gln	Tyr	Gly	Ile	Ala	Ile	Ala	Tyr	Ser	Ile	
			180					185					190			
gga	atg	gga	acc	ttt	gcg	gca	ttc	atc	ggc	acg	tgt	att	caa	cac	gat	624
Gly	Met	Gly	Thr	Phe	Ala	Ala	Phe	Ile	Gly	Thr	Cys	Ile	Gln	His	Asp	
		195					200					205				
gga	aat	cac	ggg	gca	ttc	gct	cag	aac	aag	tta	ctc	aac	aag	ttg	gct	672
Gly	Asn	His	Gly	Ala	Phe	Ala	Gln	Asn	Lys	Leu	Leu	Asn	Lys	Leu	Ala	
	210					215					220					
ggg	tgg	acg	ttg	gat	atg	att	ggg	gcg	agt	gcg	ttt	acg	tgg	gag	ctt	720
Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Phe	Thr	Trp	Glu	Leu	
225					230					235					240	
cag	cac	atg	ctg	ggg	cat	cat	cca	tat	acg	aat	gtg	ttg	gat	ggg	gtg	768
Gln	His	Met	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Val	Leu	Asp	Gly	Val	
				245					250					255		
gag	gag	gag	agg	aag	gag	agg	ggg	gag	gat	gtt	gct	ttg	gaa	gaa	aag	816
Glu	Glu	Glu	Arg	Lys	Glu	Arg	Gly	Glu	Asp	Val	Ala	Leu	Glu	Glu	Lys	
			260					265					270			
gat	cag	gat	ttt	gaa	gtt	gcc	aca	tcc	gga	cga	tta	tat	cat	att	gat	864

Asp	Gln	Asp	Phe	Glu	Val	Ala	Thr	Ser	Gly	Arg	Leu	Tyr	His	Ile	Asp	
	275						280					285				
gcc	aat	gta	cgt	tat	ggg	tcg	gta	tgg	aat	gtc	atg	agg	ttt	tgg	gct	912
Ala	Asn	Val	Arg	Tyr	Gly	Ser	Val	Trp	Asn	Val	Met	Arg	Phe	Trp	Ala	
	290					295				300						
atg	aag	gtc	att	acg	atg	gga	tat	atg	atg	gga	tta	cca	atc	tac	ttt	960
Met	Lys	Val	Ile	Thr	Met	Gly	Tyr	Met	Met	Gly	Leu	Pro	Ile	Tyr	Phe	
305					310					315					320	
cat	gga	gta	ctg	agg	gga	gtt	gga	ttg	ttt	gtt	att	ggg	cat	ttg	gcg	1008
His	Gly	Val	Leu	Arg	Gly	Val	Gly	Leu	Phe	Val	Ile	Gly	His	Leu	Ala	
				325					330					335		
tgt	gga	gag	ttg	ttg	gcg	acg	atg	ttt	att	gtg	aat	cac	gtc	att	gag	1056
Cys	Gly	Glu	Leu	Leu	Ala	Thr	Met	Phe	Ile	Val	Asn	His	Val	Ile	Glu	
			340					345					350			
ggg	gtg	agt	tat	gga	acg	aag	gat	ttg	gtt	ggg	ggg	gcg	agt	cat	gta	1104
Gly	Val	Ser	Tyr	Gly	Thr	Lys	Asp	Leu	Val	Gly	Gly	Ala	Ser	His	Val	
		355					360					365				
gat	gag	aag	aag	att	gtc	aag	cca	acg	act	gta	ttg	gga	gat	aca	cca	1152
Asp	Glu	Lys	Lys	Ile	Val	Lys	Pro	Thr	Thr	Val	Leu	Gly	Asp	Thr	Pro	
	370					375					380					
atg	gta	aag	act	cgc	gag	gag	gca	ttg	aaa	agc	aac	agc	aat	aac	aac	1200
Met	Val	Lys	Thr	Arg	Glu	Glu	Ala	Leu	Lys	Ser	Asn	Ser	Asn	Asn	Asn	
385					390					395					400	
aag	aag	aag	gga	gag	aag	aac	tcg	gta	cca	tcc	gtt	cca	ttc	aac	gac	1248
Lys	Lys	Lys	Gly	Glu	Lys	Asn	Ser	Val	Pro	Ser	Val	Pro	Phe	Asn	Asp	
			405					410					415			
tgg	gca	gca	gtc	caa	tgc	cag	acc	tcc	gtg	aat	tgg	tct	cca	ggc	tca	1296
Trp	Ala	Ala	Val	Gln	Cys	Gln	Thr	Ser	Val	Asn	Trp	Ser	Pro	Gly	Ser	
			420					425					430			
tgg	ttc	tgg	aat	cac	ttt	tct	ggg	gga	ctc	tct	cat	cag	att	gag	cat	1344
Trp	Phe	Trp	Asn	His	Phe	Ser	Gly	Gly	Leu	Ser	His	Gln	Ile	Glu	His	
		435					440					445				
cac	ttg	ttc	ccc	agc	att	tgt	cat	aca	aac	tac	tgt	cat	atc	cag	gat	1392
His	Leu	Phe	Pro	Ser	Ile	Cys	His	Thr	Asn	Tyr	Cys	His	Ile	Gln	Asp	
	450					455					460					
gtt	gtg	gag	agt	acg	tgt	gct	gag	tac	gga	gtt	ccg	tat	cag	agt	gag	1440
Val	Val	Glu	Ser	Thr	Cys	Ala	Glu	Tyr	Gly	Val	Pro	Tyr	Gln	Ser	Glu	
465					470					475					480	
agt	aat	ttg	ttt	gtt	gct	tat	gga	aag	atg	att	agt	cat	ttg	aag	ttt	1488
Ser	Asn	Leu	Phe	Val	Ala	Tyr	Gly	Lys	Met	Ile	Ser	His	Leu	Lys	Phe	
			485					490					495			
ttg	ggg	aaa	gcc	aag	tgt	gag	tag									1512
Leu	Gly	Lys	Ala	Lys	Cys	Glu										
			500													

<210> 16

<211> 503

<212> PRT

<213> *Thalassiosira pseudonana*

<400> 16

Met	Cys	Asn	Gly	Asn	Leu	Pro	Ala	Ser	Thr	Ala	Gln	Leu	Lys	Ser	Thr
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Ser	Lys	Pro	Gln	Gln	Gln	His	Glu	His	Arg	Thr	Ile	Ser	Lys	Ser	Glu
			20					25					30		

Leu	Ala	Gln	His	Asn	Thr	Pro	Lys	Ser	Ala	Trp	Cys	Ala	Val	His	Ser
		35					40					45			

Thr	Pro	Ala	Thr	Asp	Pro	Ser	His	Ser	Asn	Asn	Lys	Gln	His	Ala	His
	50					55					60				

Leu	Val	Leu	Asp	Ile	Thr	Asp	Phe	Ala	Ser	Arg	His	Pro	Gly	Gly	Asp
65					70					75					80

Leu	Ile	Leu	Leu	Ala	Ser	Gly	Lys	Asp	Ala	Ser	Val	Leu	Phe	Glu	Thr
				85					90					95	

Tyr	His	Pro	Arg	Gly	Val	Pro	Thr	Ser	Leu	Ile	Gln	Lys	Leu	Gln	Ile
			100					105					110		

Gly	Val	Met	Glu	Glu	Glu	Ala	Phe	Arg	Asp	Ser	Phe	Tyr	Ser	Trp	Thr
		115					120					125			

Asp	Ser	Asp	Phe	Tyr	Thr	Val	Leu	Lys	Arg	Arg	Val	Val	Glu	Arg	Leu
	130					135					140				

Glu	Glu	Arg	Gly	Leu	Asp	Arg	Arg	Gly	Ser	Lys	Glu	Ile	Trp	Ile	Lys
145					150					155					160

Ala	Leu	Phe	Leu	Leu	Val	Gly	Phe	Trp	Tyr	Cys	Leu	Tyr	Lys	Met	Tyr
				165					170					175	

Thr	Thr	Ser	Asp	Ile	Asp	Gln	Tyr	Gly	Ile	Ala	Ile	Ala	Tyr	Ser	Ile
			180					185					190		

Gly	Met	Gly	Thr	Phe	Ala	Ala	Phe	Ile	Gly	Thr	Cys	Ile	Gln	His	Asp
		195					200					205			

Gly	Asn	His	Gly	Ala	Phe	Ala	Gln	Asn	Lys	Leu	Leu	Asn	Lys	Leu	Ala
	210					215					220				

Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Phe Thr Trp Glu Leu
 225 230 235 240

Gln His Met Leu Gly His His Pro Tyr Thr Asn Val Leu Asp Gly Val
 245 250 255

Glu Glu Glu Arg Lys Glu Arg Gly Glu Asp Val Ala Leu Glu Glu Lys
 260 265 270

Asp Gln Asp Phe Glu Val Ala Thr Ser Gly Arg Leu Tyr His Ile Asp
 275 280 285

Ala Asn Val Arg Tyr Gly Ser Val Trp Asn Val Met Arg Phe Trp Ala
 290 295 300

Met Lys Val Ile Thr Met Gly Tyr Met Met Gly Leu Pro Ile Tyr Phe
 305 310 315 320

His Gly Val Leu Arg Gly Val Gly Leu Phe Val Ile Gly His Leu Ala
 325 330 335

Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn His Val Ile Glu
 340 345 350

Gly Val Ser Tyr Gly Thr Lys Asp Leu Val Gly Gly Ala Ser His Val
 355 360 365

Asp Glu Lys Lys Ile Val Lys Pro Thr Thr Val Leu Gly Asp Thr Pro
 370 375 380

Met Val Lys Thr Arg Glu Glu Ala Leu Lys Ser Asn Ser Asn Asn Asn
 385 390 395 400

Lys Lys Lys Gly Glu Lys Asn Ser Val Pro Ser Val Pro Phe Asn Asp
 405 410 415

Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser Pro Gly Ser
 420 425 430

Trp Phe Trp Asn His Phe Ser Gly Gly Leu Ser His Gln Ile Glu His
 435 440 445

His Leu Phe Pro Ser Ile Cys His Thr Asn Tyr Cys His Ile Gln Asp
 450 455 460

Val Val Glu Ser Thr Cys Ala Glu Tyr Gly Val Pro Tyr Gln Ser Glu
 465 470 475 480

ctg aac ttg gcc gtg gga cga gac tgc acc gcg ctc atc gaa tcc tat 432
Leu Asn Leu Ala Val Gly Arg Asp Cys Thr Ala Leu Ile Glu Ser Tyr

130	135	140	
cac ttg cga cca gag gtg gcg acg gct cgt ttc aga atg ctg ccc aaa			480
His Leu Arg Pro Glu Val Ala Thr Ala Arg Phe Arg Met Leu Pro Lys			
145	150	155	160
ctc gag gat ttt ccc gtc gag gcc gtg ccc aag tcc ccg aga ccg aac			528
Leu Glu Asp Phe Pro Val Glu Ala Val Pro Lys Ser Pro Arg Pro Asn			
	165	170	175
gat tcg ccg tta tac aac aac att cgc aac cga gtc cgc gaa gag ctc			576
Asp Ser Pro Leu Tyr Asn Asn Ile Arg Asn Arg Val Arg Glu Glu Leu			
	180	185	190
ttc cca gag gag gga aag aat atg cac aga cag gcc gcc gac cac gcc			624
Phe Pro Glu Glu Gly Lys Asn Met His Arg Gln Gly Gly Asp His Gly			
	195	200	205
gac ggt gac gat tct ggg ttt cgc cgc ctt ttg ctt atg ccg tgt acc			672
Asp Gly Asp Asp Ser Gly Phe Arg Arg Leu Leu Met Pro Cys Thr			
	210	215	220
tat tcc ctt ccg ggg gtt cct ttc cgg ctg cct cct cgg gtc tcg cgg			720
Tyr Ser Leu Pro Gly Val Pro Phe Arg Leu Pro Pro Arg Val Ser Arg			
	225	230	235
ggg cgt gga ttg gtc tca cga ttc agg cac tgc gcc aac cac gcc gcg			768
Gly Arg Gly Leu Val Ser Arg Phe Arg His Cys Ala Asn His Gly Ala			
	245	250	255
atg tct cct tcg ccg gcc gtt aac ggc gtc ctc ggt ttg acg aac gat			816
Met Ser Pro Ser Pro Ala Val Asn Gly Val Leu Gly Leu Thr Asn Asp			
	260	265	270
ctc atc ggc ggc tcg tcc ttg atg tgg aga tat cac cac caa gtc agc			864
Leu Ile Gly Gly Ser Ser Leu Met Trp Arg Tyr His His Gln Val Ser			
	275	280	285
cac cac att cat tgc aac gac aac gcc atg gat caa gac gtg tac acg			912
His His Ile His Cys Asn Asp Asn Ala Met Asp Gln Asp Val Tyr Thr			
	290	295	300
gcg atg cca tta ttg cgt ttc gac gct cgc cgg ccc aag tcc tgg tac			960
Ala Met Pro Leu Leu Arg Phe Asp Ala Arg Arg Pro Lys Ser Trp Tyr			
	305	310	315
cat cgc ttc cag cag tgg tac atg ttt tta gcg ttc ccg ttg ttg cag			1008
His Arg Phe Gln Gln Trp Tyr Met Phe Leu Ala Phe Pro Leu Leu Gln			
	325	330	335
gtt gcc ttc caa gtc gga gac att gcc gca ctg ttc acg cgt gat acc			1056
Val Ala Phe Gln Val Gly Asp Ile Ala Ala Leu Phe Thr Arg Asp Thr			
	340	345	350
gaa ggc gct aag ctt cac ggg gcg acg acg tgg gag ctt acc acg gtt			1104
Glu Gly Ala Lys Leu His Gly Ala Thr Thr Trp Glu Leu Thr Thr Val			
	355	360	365
gtc ctc ggt aag att gtg cac ttc ggt ctt ttg ttg ggg ccg ttg atg			1152
Val Leu Gly Lys Ile Val His Phe Gly Leu Leu Leu Gly Pro Leu Met			
	370	375	380
aac cac gcg gtg agt tct gtt ttg ctg ggg atc gtc ggt ttc atg gcg			1200
Asn His Ala Val Ser Ser Val Leu Leu Gly Ile Val Gly Phe Met Ala			

385	390	395	400	
tgc caa ggt ata gtt ctg gcg tgc acg ttt gct gtg agt cac aat gtc				1248
Cys Gln Gly Ile Val Leu Ala Cys Thr Phe Ala Val Ser His Asn Val				
	405	410	415	
gcg gag gcg aag ata cct gag gac acc gga gga gaa gcc tgg gag aga				1296
Ala Glu Ala Lys Ile Pro Glu Asp Thr Gly Gly Glu Ala Trp Glu Arg				
	420	425	430	
gat tgg ggt gtc cag cag ttg gtg act agc gcc gac tgg ggt gga aag				1344
Asp Trp Gly Val Gln Gln Leu Val Thr Ser Ala Asp Trp Gly Gly Lys				
	435	440	445	
ata ggt aac ttc ttc acg ggt ggc ctc aac ttg caa gtt gag cac cac				1392
Ile Gly Asn Phe Phe Thr Gly Gly Leu Asn Leu Gln Val Glu His His				
	450	455	460	
ttg ttt ccg gcg att tgc ttc gtc cac tac ccg gac atc gcg aag atc				1440
Leu Phe Pro Ala Ile Cys Phe Val His Tyr Pro Asp Ile Ala Lys Ile				
	465	470	475	480
gtg aag gaa gaa gcg gcc aag ctc aac atc cct tac gcg tct tac agg				1488
Val Lys Glu Glu Ala Ala Lys Leu Asn Ile Pro Tyr Ala Ser Tyr Arg				
	485	490	495	
act ctt cct ggt att ttc gtc caa ttc tgg aga ttt atg aag gac atg				1536
Thr Leu Pro Gly Ile Phe Val Gln Phe Trp Arg Phe Met Lys Asp Met				
	500	505	510	
ggc acg gct gag caa att ggt gaa gtt cca ttg ccg aag att ccc aac				1584
Gly Thr Ala Glu Gln Ile Gly Glu Val Pro Leu Pro Lys Ile Pro Asn				
	515	520	525	
ccg cag ctc gcg ccg aag ctc gct tag				1611
Pro Gln Leu Ala Pro Lys Leu Ala				
	530	535		
<210> 18				
<211> 536				
<212> PRT				
<213> <i>Ostreococcus tauri</i>				
<400> 18				
Met Tyr Leu Gly Arg Gly Arg Leu Glu Ser Gly Thr Thr Arg Gly Met				
1	5	10	15	
Met Arg Thr His Ala Arg Arg Pro Ser Thr Thr Ser Asn Pro Cys Ala				
	20	25	30	
Arg Ser Arg Val Arg Lys Thr Thr Glu Arg Ser Leu Ala Arg Val Arg				
	35	40	45	

Arg Ser Thr Ser Glu Lys Gly Ser Ala Leu Val Leu Glu Arg Glu Ser
50 55 60

Glu Arg Glu Lys Glu Glu Gly Gly Lys Ala Arg Ala Glu Gly Leu Arg
65 70 75 80

Phe Gln Arg Pro Asp Val Ala Ala Pro Gly Gly Ala Asp Pro Trp Asn
85 90 95

Asp Glu Lys Trp Thr Lys Thr Lys Trp Thr Val Phe Arg Asp Val Ala
100 105 110

Tyr Asp Leu Asp Pro Phe Phe Ala Arg His Pro Gly Gly Asp Trp Leu
115 120 125

Leu Asn Leu Ala Val Gly Arg Asp Cys Thr Ala Leu Ile Glu Ser Tyr
130 135 140

His Leu Arg Pro Glu Val Ala Thr Ala Arg Phe Arg Met Leu Pro Lys
145 150 155 160

Leu Glu Asp Phe Pro Val Glu Ala Val Pro Lys Ser Pro Arg Pro Asn
165 170 175

Asp Ser Pro Leu Tyr Asn Asn Ile Arg Asn Arg Val Arg Glu Glu Leu
180 185 190

Phe Pro Glu Glu Gly Lys Asn Met His Arg Gln Gly Gly Asp His Gly
195 200 205

Asp Gly Asp Asp Ser Gly Phe Arg Arg Leu Leu Leu Met Pro Cys Thr
210 215 220

Tyr Ser Leu Pro Gly Val Pro Phe Arg Leu Pro Pro Arg Val Ser Arg
225 230 235 240

Gly Arg Gly Leu Val Ser Arg Phe Arg His Cys Ala Asn His Gly Ala
245 250 255

Met Ser Pro Ser Pro Ala Val Asn Gly Val Leu Gly Leu Thr Asn Asp
260 265 270

Leu Ile Gly Gly Ser Ser Leu Met Trp Arg Tyr His His Gln Val Ser
275 280 285

His His Ile His Cys Asn Asp Asn Ala Met Asp Gln Asp Val Tyr Thr
290 295 300

Ala Met Pro Leu Leu Arg Phe Asp Ala Arg Arg Pro Lys Ser Trp Tyr
 305 310 315 320

His Arg Phe Gln Gln Trp Tyr Met Phe Leu Ala Phe Pro Leu Leu Gln
 325 330 335

Val Ala Phe Gln Val Gly Asp Ile Ala Ala Leu Phe Thr Arg Asp Thr
 340 345 350

Glu Gly Ala Lys Leu His Gly Ala Thr Thr Trp Glu Leu Thr Thr Val
 355 360 365

Val Leu Gly Lys Ile Val His Phe Gly Leu Leu Leu Gly Pro Leu Met
 370 375 380

Asn His Ala Val Ser Ser Val Leu Leu Gly Ile Val Gly Phe Met Ala
 385 390 395 400

Cys Gln Gly Ile Val Leu Ala Cys Thr Phe Ala Val Ser His Asn Val
 405 410 415

Ala Glu Ala Lys Ile Pro Glu Asp Thr Gly Gly Glu Ala Trp Glu Arg
 420 425 430

Asp Trp Gly Val Gln Gln Leu Val Thr Ser Ala Asp Trp Gly Gly Lys
 435 440 445

Ile Gly Asn Phe Phe Thr Gly Gly Leu Asn Leu Gln Val Glu His His
 450 455 460

Leu Phe Pro Ala Ile Cys Phe Val His Tyr Pro Asp Ile Ala Lys Ile
 465 470 475 480

Val Lys Glu Glu Ala Ala Lys Leu Asn Ile Pro Tyr Ala Ser Tyr Arg
 485 490 495

Thr Leu Pro Gly Ile Phe Val Gln Phe Trp Arg Phe Met Lys Asp Met
 500 505 510

Gly Thr Ala Glu Gln Ile Gly Glu Val Pro Leu Pro Lys Ile Pro Asn
 515 520 525

Pro Gln Leu Ala Pro Lys Leu Ala
 530 535

<210> 19

<211> 1548

<212> DNA

<213> Thraustochytrium

<220>

<221> CDS

<222> (1)..(1548)

<223> Delta-4 desaturase

<400> 19

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Met	Thr	Val	Gly	Phe	Asp	Glu	Thr	Val	Thr	Met	Asp	Thr	Val	Arg	Asn	
1			5						10					15		

cac	aac	atg	ccg	gac	gac	gcc	tgg	tgc	gcg	atc	cac	ggc	acc	gtg	tac	96
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr	
			20					25					30			

gac	atc	acc	aag	ttc	agc	aag	gtg	cac	ccc	ggc	ggg	gac	atc	atc	atg	144
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met	
		35					40					45				

ctg	gcc	gct	ggc	aag	gag	gcc	acc	atc	ctg	ttc	gag	acc	tac	cac	atc	192
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile	
	50					55					60					

aag	ggc	gtc	ccg	gac	gcg	gtg	ctg	cgc	aag	tac	aag	gtc	ggc	aag	ctc	240
Lys	Gly	Val	Pro	Asp	Ala	Val	Leu	Arg	Lys	Tyr	Lys	Val	Gly	Lys	Leu	
65					70				75					80		

ccc	cag	ggc	aag	aag	ggc	gaa	acg	agc	cac	atg	ccc	acc	ggg	ctc	gac	288
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp	
			85						90					95		

tcg	gcc	tcc	tac	tac	tcg	tgg	gac	agc	gag	ttt	tac	agg	gtg	ctc	cgc	336
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg	
			100					105					110			

gag	cgc	gtc	gcc	aag	aag	ctg	gcc	gag	ccc	ggc	ctc	atg	cag	cgc	gcg	384
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala	
		115				120						125				

cgc	atg	gag	ctc	tgg	gcc	aag	gcg	atc	ttc	ctc	ctg	gca	ggg	ttc	tgg	432
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp	
	130					135						140				

ggc	tcc	ctt	tac	gcc	atg	tgc	gtg	cta	gac	ccg	cac	ggc	ggg	gcc	atg	480
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met	
145					150					155				160		

gta	gcc	gcc	gtt	acg	ctc	ggc	gtg	ttc	gct	gcc	ttt	gtc	gga	act	tgc	528
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys	
				165				170						175		

atc	cag	cac	gac	ggc	agc	cac	ggc	gcc	ttc	tcc	aag	tcg	cga	ttc	atg	576
Ile	Gln	His	Asp	Gly	Ser	His	Gly	Ala	Phe	Ser	Lys	Ser	Arg	Phe	Met	
			180					185					190			

aac aag gcg gcg ggc tgg acc ctc gac atg atc ggc gcg agt gcg atg Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met 195 200 205	624
acc tgg gag atg cag cac gtt ctt ggc cac cac ccg tac acc aac ctc Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu 210 215 220	672
atc gag atg gag aac ggt ttg gcc aag gtc aag ggc gcc gac gtc gac Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp 225 230 235 240	720
ccg aag aag gtc gac cag gag agc gac ccg gac gtc ttc agt acg tac Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr 245 250 255	768
ccg atg ctt cgc ctg cac ccg tgg cac cgc cag ccg ttt tac cac aag Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys 260 265 270	816
ttc cag cac ctg tac gcc ccg ttt atc ttt ggg tct atg acg att aac Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn 275 280 285	864
aag gtg att tcc cag gat gtc ggg gtt gtg ctg cgc aag cgc ctg ttc Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe 290 295 300	912
cag atc gac gcc aac tgc ccg tat ggc agc ccc tgg tac gtg gcc cgc Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg 305 310 315 320	960
ttc tgg atc atg aag ctc ctc acc acg ctc tac atg gtg gcg ctt ccc Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro 325 330 335	1008
atg tac atg cag ggg cct gct cag ggc ttg aag ctt ttc ttc atg gcc Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala 340 345 350	1056
cac ttc acc tgc gga gag gtc ctc gcc acc atg ttt att gtc aac cac His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His 355 360 365	1104
atc atc gag ggc gtc agc tac gct tcc aag gac gcg gtc aag ggc gtc Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val 370 375 380	1152
atg gct ccg ccg cgc act gtg cac ggt gtc acc ccg atg cag gtg acg Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr 385 390 395 400	1200
caa aag gcg ctc agt gcg gcc gag tcg gcc aag tcg gac gcc gac aag Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys 405 410 415	1248
acg acc atg atc ccc ctc aac gac tgg gcc gct gtg cag tgc cag acc Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr 420 425 430	1296
tct gtg aac tgg gct gtc ggg tcg tgg ttt tgg aac cac ttt tcg ggc Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly 435 440 445	1344

ggc ctc aac cac cag att gag cac cac tgc ttc ccc caa aac ccc cac 1392
 Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
 450 455 460

acg gtc aac gtc tac atc tcg ggc atc gtc aag gag acc tgc gaa gaa 1440
 Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
 465 470 475 480

tac ggc gtg ccg tac cag gct gag atc agc ctc ttc tct gcc tat ttc 1488
 Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
 485 490 495

aag atg ctg tcg cac ctc cgc acg ctc ggc aac gag gac ctc acg gcc 1536
 Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
 500 505 510

tgg tcc acg tga 1548
 Trp Ser Thr
 515

<210> 20

<211> 515

<212> PRT

<213> Thraustochytrium

<400> 20

Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
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His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
 20 25 30

Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
 35 40 45

Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
 50 55 60

Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
 65 70 75 80

Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
 85 90 95

Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
 100 105 110

Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
 115 120 125

Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
 130 135 140

Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
 145 150 155 160

Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
 165 170 175

Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
 180 185 190

Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
 195 200 205

Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
 210 215 220

Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
 225 230 235 240

Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
 245 250 255

Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
 260 265 270

Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn
 275 280 285

Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
 290 295 300

Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
 305 310 315 320

Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
 325 330 335

Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
 340 345 350

His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
 355 360 365

Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
 370 375 380

Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385 390 395 400

Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys
405 410 415

Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
420 425 430

Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
435 440 445

Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
450 455 460

Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465 470 475 480

Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
485 490 495

Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
500 505 510

Trp Ser Thr
515

<210> 21

<211> 1626

<212> DNA

<213> Euglena gracilis

<220>

<221> CDS

<222> (1)..(1626)

<223> Delta-4 desaturase

<400> 21

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aac ggc aag ccg gag aac gga gcc acc cct gag aac gga gcg aag ccg 96

Asn	Gly	Lys	Pro	Glu	Asn	Gly	Ala	Thr	Pro	Glu	Asn	Gly	Ala	Lys	Pro	
			20					25						30		
caa	cct	tgc	gag	aac	ggc	acg	gtg	gaa	aag	cga	gag	aat	gac	acc	gcc	144
Gln	Pro	Cys	Glu	Asn	Gly	Thr	Val	Glu	Lys	Arg	Glu	Asn	Asp	Thr	Ala	
		35					40					45				
aac	gtt	cgg	ccc	acc	cgt	cca	gct	gga	ccc	ccg	ccg	gcc	acg	tac	tac	192
Asn	Val	Arg	Pro	Thr	Arg	Pro	Ala	Gly	Pro	Pro	Pro	Ala	Thr	Tyr	Tyr	
	50					55				60						
gac	tcc	ctg	gca	gtg	tcg	ggg	cag	ggc	aag	gag	cgg	ctg	ttc	acc	acc	240
Asp	Ser	Leu	Ala	Val	Ser	Gly	Gln	Gly	Lys	Glu	Arg	Leu	Phe	Thr	Thr	
65					70				75					80		
gat	gag	gtg	agg	cgg	cac	atc	ctc	ccc	acc	gat	ggc	tgg	ctg	acg	tgc	288
Asp	Glu	Val	Arg	Arg	His	Ile	Leu	Pro	Thr	Asp	Gly	Trp	Leu	Thr	Cys	
				85				90						95		
cac	gaa	gga	gtc	tac	gat	gtc	act	gat	ttc	ctt	gcc	aag	cac	cct	ggt	336
His	Glu	Gly	Val	Tyr	Asp	Val	Thr	Asp	Phe	Leu	Ala	Lys	His	Pro	Gly	
			100					105					110			
ggc	ggt	gtc	atc	acg	ctg	ggc	ctt	gga	agg	gac	tgc	aca	atc	ctc	atc	384
Gly	Gly	Val	Ile	Thr	Leu	Gly	Leu	Gly	Arg	Asp	Cys	Thr	Ile	Leu	Ile	
		115				120					125					
gag	tca	tac	cac	cct	gct	ggg	cgc	ccg	gac	aag	gtg	atg	gag	aag	tac	432
Glu	Ser	Tyr	His	Pro	Ala	Gly	Arg	Pro	Asp	Lys	Val	Met	Glu	Lys	Tyr	
	130					135					140					
cgc	att	ggt	acg	ctg	cag	gac	ccc	aag	acg	ttc	tat	gct	tgg	gga	gag	480
Arg	Ile	Gly	Thr	Leu	Gln	Asp	Pro	Lys	Thr	Phe	Tyr	Ala	Trp	Gly	Glu	
145					150				155					160		
tcc	gat	ttc	tac	cct	gag	ttg	aag	cgc	cgg	gcc	ctt	gca	agg	ctg	aag	528
Ser	Asp	Phe	Tyr	Pro	Glu	Leu	Lys	Arg	Arg	Ala	Leu	Ala	Arg	Leu	Lys	
				165				170						175		
gag	gct	ggt	cag	gcg	cgg	cgc	ggc	ggc	ctt	ggg	gtg	aag	gcc	ctc	ctg	576
Glu	Ala	Gly	Gln	Ala	Arg	Arg	Gly	Gly	Leu	Gly	Val	Lys	Ala	Leu	Leu	
			180				185						190			
gtg	ctc	acc	ctc	ttc	ttc	gtg	tcg	tgg	tac	atg	tgg	gtg	gcc	cac	aag	624
Val	Leu	Thr	Leu	Phe	Phe	Val	Ser	Trp	Tyr	Met	Trp	Val	Ala	His	Lys	
		195				200						205				
tcc	ttc	ctc	tgg	gcc	gcc	gtc	tgg	ggc	ttc	gcc	ggc	tcc	cac	gtc	ggg	672
Ser	Phe	Leu	Trp	Ala	Ala	Val	Trp	Gly	Phe	Ala	Gly	Ser	His	Val	Gly	
	210					215					220					
ctg	agc	atc	cag	cac	gat	ggc	aac	cac	ggc	gcg	ttc	agc	cgc	aac	aca	720
Leu	Ser	Ile	Gln	His	Asp	Gly	Asn	His	Gly	Ala	Phe	Ser	Arg	Asn	Thr	
225					230				235					240		
ctg	gtg	aac	cgc	ctg	gcg	ggg	tgg	ggc	atg	gac	ttg	atc	ggc	gcg	tcg	768
Leu	Val	Asn	Arg	Leu	Ala	Gly	Trp	Gly	Met	Asp	Leu	Ile	Gly	Ala	Ser	
				245				250					255			
tcc	acg	gtg	tgg	gag	tac	cag	cac	gtc	atc	ggc	cac	cac	cag	tac	acc	816
Ser	Thr	Val	Trp	Glu	Tyr	Gln	His	Val	Ile	Gly	His	His	Gln	Tyr	Thr	
			260					265					270			

aac ctc gtg tcg gac acg cta ttc agt ctg cct gag aac gat ccg gac	864
Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp	
275 280 285	
gtc ttc tcc agc tac ccg ctg atg cgc atg cac ccg gat acg gcg tgg	912
Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp	
290 295 300	
cag ccg cac cac cgc ttc cag cac ctg ttc gcg ttc cca ctg ttc gcc	960
Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala	
305 310 315 320	
ctg atg aca atc agc aag gtg ctg acc agc gat ttc gct gtc tgc ctc	1008
Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu	
325 330 335	
agc atg aag aag ggg tcc atc gac tgc tcc tcc agg ctc gtc cca ctg	1056
Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu	
340 345 350	
gag ggg cag ctg ctg ttc tgg ggg gcc aag ctg gcg aac ttc ctg ttg	1104
Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu	
355 360 365	
cag att gtg ttg cca tgc tac ctc cac ggg aca gct atg ggc ctg gcc	1152
Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala	
370 375 380	
ctc ttc tct gtt gct cac ctt gtg tcg ggg gag tac ctc gcg atc tgc	1200
Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys	
385 390 395 400	
ttc atc atc aac cac atc agc gag tct tgt gag ttt atg aat aca agc	1248
Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser	
405 410 415	
ttt caa acc gcc gcc cgg agg aca gag atg ctt cag gca gca cat cag	1296
Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln	
420 425 430	
gca gcg gag gcc aag aag gtg aag ccc acc cct cca ccg aac gat tgg	1344
Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Pro Asn Asp Trp	
435 440 445	
gct gtg aca cag gtc caa tgc tgc gtg aat tgg aga tca ggt ggc gtg	1392
Ala Val Thr Gln Val Gln Cys Cys Val Asn Trp Arg Ser Gly Gly Val	
450 455 460	
ttg gcc aat cac ctc tct gga ggc ttg aac cac cag atc gag cat cat	1440
Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His	
465 470 475 480	
ctg ttc ccc agc atc tcg cat gcc aac tac ccc acc atc gcc cct gtt	1488
Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val	
485 490 495	
gtg aag gag gtg tgc gag gag tac ggg ttg ccg tac aag aat tac gtc	1536
Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val	
500 505 510	
acg ttc tgg gat gca gtc tgt ggc atg gtt cag cac ctc cgg ttg atg	1584

Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
 515 520 525

ggt gct cca ccg gtg cca acg aac ggg gac aaa aag tca taa 1626
 Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
 530 535 540

<210> 22

<211> 541

<212> PRT

<213> Euglena gracilis

<400> 22

Met Leu Val Leu Phe Gly Asn Phe Tyr Val Lys Gln Tyr Ser Gln Lys
 1 5 10 15

Asn Gly Lys Pro Glu Asn Gly Ala Thr Pro Glu Asn Gly Ala Lys Pro
 20 25 30

Gln Pro Cys Glu Asn Gly Thr Val Glu Lys Arg Glu Asn Asp Thr Ala
 35 40 45

Asn Val Arg Pro Thr Arg Pro Ala Gly Pro Pro Pro Ala Thr Tyr Tyr
 50 55 60

Asp Ser Leu Ala Val Ser Gly Gln Gly Lys Glu Arg Leu Phe Thr Thr
 65 70 75 80

Asp Glu Val Arg Arg His Ile Leu Pro Thr Asp Gly Trp Leu Thr Cys
 85 90 95

His Glu Gly Val Tyr Asp Val Thr Asp Phe Leu Ala Lys His Pro Gly
 100 105 110

Gly Gly Val Ile Thr Leu Gly Leu Gly Arg Asp Cys Thr Ile Leu Ile
 115 120 125

Glu Ser Tyr His Pro Ala Gly Arg Pro Asp Lys Val Met Glu Lys Tyr
 130 135 140

Arg Ile Gly Thr Leu Gln Asp Pro Lys Thr Phe Tyr Ala Trp Gly Glu
 145 150 155 160

Ser Asp Phe Tyr Pro Glu Leu Lys Arg Arg Ala Leu Ala Arg Leu Lys
 165 170 175

Glu Ala Gly Gln Ala Arg Arg Gly Gly Leu Gly Val Lys Ala Leu Leu
 180 185 190

Val Leu Thr Leu Phe Phe Val Ser Trp Tyr Met Trp Val Ala His Lys
 195 200 205

Ser Phe Leu Trp Ala Ala Val Trp Gly Phe Ala Gly Ser His Val Gly
 210 215 220

Leu Ser Ile Gln His Asp Gly Asn His Gly Ala Phe Ser Arg Asn Thr
 225 230 235 240

Leu Val Asn Arg Leu Ala Gly Trp Gly Met Asp Leu Ile Gly Ala Ser
 245 250 255

Ser Thr Val Trp Glu Tyr Gln His Val Ile Gly His His Gln Tyr Thr
 260 265 270

Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp
 275 280 285

Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp
 290 295 300

Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala
 305 310 315 320

Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu
 325 330 335

Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu
 340 345 350

Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu
 355 360 365

Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala
 370 375 380

Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys
 385 390 395 400

Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser
 405 410 415

Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln
 420 425 430

Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Pro Asn Asp Trp
 435 440 445

Ala Val Thr Gln Val Gln Cys Cys Val Asn Trp Arg Ser Gly Gly Val
 450 455 460

Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His
 465 470 475 480

Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val
 485 490 495

Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val
 500 505 510

Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
 515 520 525

Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
 530 535 540

<210> 23

<211> 1092

<212> DNA

<213> Phytophthora sojae

<220>

<221> CDS

<222> (1)..(1092)

<223> Omega-3 desaturase

<400> 23

atg gcg tcc aag cag gag cag ccg tac cag ttc ccg acg ctg acg gag 48
 Met Ala Ser Lys Gln Glu Gln Pro Tyr Gln Phe Pro Thr Leu Thr Glu
 1 5 10 15

atc aag cgc tcg ctg ccc agc gag tgt ttc gag gcg tcc gtg ccg ctc 96
 Ile Lys Arg Ser Leu Pro Ser Glu Cys Phe Glu Ala Ser Val Pro Leu
 20 25 30

tcg ctc tac tac acg gtg cgc tgc ctg gtg atc gcc gtg tcg ctg gcc 144
 Ser Leu Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ser Leu Ala
 35 40 45

ttc ggg ctc cac cac gcg cgc tcg ctg ccc gtg gtc gag ggc ctc tgg 192
 Phe Gly Leu His His Ala Arg Ser Leu Pro Val Val Glu Gly Leu Trp

50	55	60	
gcg ctg gac gcc gcg ctc tgc acg ggc tac gtg ctg ctg cag ggc atc Ala Leu Asp Ala Ala Leu Cys Thr Gly Tyr Val Leu Leu Gln Gly Ile 65 70 75 80			240
gtg ttc tgg ggc ttc ttc acc gtg ggc cat gac gcc ggc cac ggc gcc Val Phe Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala 85 90 95			288
ttc tcg cgc tac cac ctg ctc aac ttc gtg atc ggc acc ttc atc cac Phe Ser Arg Tyr His Leu Leu Asn Phe Val Ile Gly Thr Phe Ile His 100 105 110			336
tcg ctc atc ctg acg ccc ttc gag tcg tgg aag ctc acg cac cgc cac Ser Leu Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His 115 120 125			384
cac cac aag aac acg ggc aac atc gac cgc gac gag atc ttc tac ccg His His Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Ile Phe Tyr Pro 130 135 140			432
cag cgc aag gcc gac gac cac ccg ctc tcg cgt aac ctc atc ctg gcg Gln Arg Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala 145 150 155 160			480
ctg ggc gcc gcg tgg ttc gcc tac ctg gtc gag ggc ttc ccg ccg cgc Leu Gly Ala Ala Trp Phe Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg 165 170 175			528
aag gtc aac cac ttc aac ccg ttc gag ccg ctg ttc gtc cgc cag gtg Lys Val Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val 180 185 190			576
tcc gcc gtg gtc atc tcg ctg gcc gcg cac ttc ggc gtg gcc gcg ctg Ser Ala Val Val Ile Ser Leu Ala Ala His Phe Gly Val Ala Ala Leu 195 200 205			624
tcc atc tac ctg agc ctg cag ttc ggc ttc aag acc atg gct atc tac Ser Ile Tyr Leu Ser Leu Gln Phe Gly Phe Lys Thr Met Ala Ile Tyr 210 215 220			672
tac tac ggg ccc gtg ttc gtg ttc ggc agc atg ctg gtc atc acc acc Tyr Tyr Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr 225 230 235 240			720
ttc ctg cac cac aac gac gag gag acc ccc tgg tac gcc gac tcg gag Phe Leu His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu 245 250 255			768
tgg acc tac gtc aag ggc aac ctc tcg tcg gtc gac cgc tcc tac ggc Trp Thr Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly 260 265 270			816
gcg ctc atc gac aac ctg agc cac aac atc ggc acg cac cag atc cac Ala Leu Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His 275 280 285			864
cac ctc ttc ccc atc atc ccg cac tat aag ctc aag cgc gcc acc gag His Leu Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Arg Ala Thr Glu 290 295 300			912
gcc ttc cac cag gcg ttc ccc gag ctc gtg cgc aag agc gac gag ccc Ala Phe His Gln Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro 305 310 315 320			960

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305          310          315          320
atc att aag gcc ttc ttc cgc gtc ggc cgc ctc tac gcc aac tac ggc      1008
Ile Ile Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly
          325          330          335

gtc gtg gac tcg gac gcc aag ctc ttc acg ctc aag gag gcc aag gcc      1056
Val Val Asp Ser Asp Ala Lys Leu Phe Thr Leu Lys Glu Ala Lys Ala
          340          345          350

gtg tcc gag gcg gcg acc aag act aag gcc aac tga      1092
Val Ser Glu Ala Ala Thr Lys Thr Lys Ala Asn
          355          360

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<210> 24

<211> 363

<212> PRT

<213> *Phytophthora sojae*

<400> 24

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Met Ala Ser Lys Gln Glu Gln Pro Tyr Gln Phe Pro Thr Leu Thr Glu
1          5          10          15

Ile Lys Arg Ser Leu Pro Ser Glu Cys Phe Glu Ala Ser Val Pro Leu
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Ser Leu Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ser Leu Ala
          35          40          45

Phe Gly Leu His His Ala Arg Ser Leu Pro Val Val Glu Gly Leu Trp
          50          55          60

Ala Leu Asp Ala Ala Leu Cys Thr Gly Tyr Val Leu Leu Gln Gly Ile
65          70          75          80

Val Phe Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala
          85          90          95

Phe Ser Arg Tyr His Leu Leu Asn Phe Val Ile Gly Thr Phe Ile His
          100          105          110

Ser Leu Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His
          115          120          125

His His Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Ile Phe Tyr Pro
          130          135          140

Gln Arg Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala

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<213> Phytophthora sojae

<220>

<221> CDS

<222> (1)..(1197)

<223> Delta-12 desaturase

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Met	Ala	Ile	Leu	Asn	Pro	Glu	Ala	Asp	Ser	Ala	Ala	Asn	Leu	Ala	Thr	
1				5					10					15		

gac	agc	gag	gcc	aag	cag	cgc	cag	ctc	gcg	gag	gcc	ggc	tac	acg	cac	96
Asp	Ser	Glu	Ala	Lys	Gln	Arg	Gln	Leu	Ala	Glu	Ala	Gly	Tyr	Thr	His	
			20					25					30			

gtg	gag	ggc	gcg	ccg	gcg	cca	ctg	ccg	ctg	gag	ctg	ccg	cac	ttc	tcg	144
Val	Glu	Gly	Ala	Pro	Ala	Pro	Leu	Pro	Leu	Glu	Leu	Pro	His	Phe	Ser	
		35					40					45				

ctg	cgc	gac	ctg	cgc	gcc	gcc	atc	ccc	aag	cac	tgc	ttc	gag	cgc	tcg	192
Leu	Arg	Asp	Leu	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Phe	Glu	Arg	Ser	
	50					55					60					

ttc	gtc	acg	tcc	acg	tac	tac	atg	atc	aag	aac	gtg	ctc	acg	tgc	gcc	240
Phe	Val	Thr	Ser	Thr	Tyr	Tyr	Met	Ile	Lys	Asn	Val	Leu	Thr	Cys	Ala	
65					70					75					80	

gcg	ctc	ttc	tac	gcg	gcc	acc	ttc	atc	gac	cgc	gcg	ggc	gcc	gcc	gcc	288
Ala	Leu	Phe	Tyr	Ala	Ala	Thr	Phe	Ile	Asp	Arg	Ala	Gly	Ala	Ala	Ala	
				85					90					95		

tac	gtg	ctg	tgg	ccc	gtg	tac	tgg	ttc	ttc	cag	ggc	agc	tac	ctc	acg	336
Tyr	Val	Leu	Trp	Pro	Val	Tyr	Trp	Phe	Phe	Gln	Gly	Ser	Tyr	Leu	Thr	
			100					105					110			

ggc	gtc	tgg	gtc	atc	gcg	cac	gag	tgt	ggc	cac	cag	gcc	tac	tgc	tcg	384
Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	Gln	Ala	Tyr	Cys	Ser	
		115					120					125				

agc	gag	gtc	gtc	aac	aac	ctc	atc	ggc	ctc	gta	ctg	cac	tcg	gcg	ctg	432
Ser	Glu	Val	Val	Asn	Asn	Leu	Ile	Gly	Leu	Val	Leu	His	Ser	Ala	Leu	
		130				135					140					

ctt	gtg	ccg	tac	cac	agc	tgg	cgc	atc	tcg	cac	cgc	aag	cac	cac	tcc	480
Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	Ile	Ser	His	Arg	Lys	His	His	Ser	
145					150					155					160	

aac	acg	ggc	agc	tgc	gag	aac	gac	gag	gtg	ttc	gtg	ccc	gtg	acc	cgc	528
Asn	Thr	Gly	Ser	Cys	Glu	Asn	Asp	Glu	Val	Phe	Val	Pro	Val	Thr	Arg	
				165				170						175		

tcg	gtg	ctc	gcc	agc	tcc	tgg	aac	gag	acg	ctc	gag	gac	tcg	ccg	ctc	576
Ser	Val	Leu	Ala	Ser	Ser	Trp	Asn	Glu	Thr	Leu	Glu	Asp	Ser	Pro	Leu	
			180					185					190			

tac	cag	ctc	tac	cgc	atc	gtg	tac	atg	ctg	gtc	gtg	ggc	tgg	atg	ccc	624
Tyr	Gln	Leu	Tyr	Arg	Ile	Val	Tyr	Met	Leu	Val	Val	Gly	Trp	Met	Pro	
		195				200						205				

ggc tac ctc ttc ttc aac gcc acg ggc ccg acc aag tac tgg ggc aag 672
 Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
 210 215 220

 tgc cgc agc cac ttc aac ccg tac tgc gcc atc tac gcc gac cgc gag 720
 Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ala Asp Arg Glu
 225 230 235 240

 cgc tgg atg atc gtg ctg agc gac atc ttc ctc gtg gcc atg ctg gcc 768
 Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Ala Met Leu Ala
 245 250 255

 gtg ctg gcc gcg ctc gtg cac acc ttc tcc ttc aac acc atg gtc aag 816
 Val Leu Ala Ala Leu Val His Thr Phe Ser Phe Asn Thr Met Val Lys
 260 265 270

 ttc tac gtc gtg ccc tac ttc atc gtc aac gcc tac ctc gtg ctt atc 864
 Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
 275 280 285

 acg tac ctg cag cac acg gac acg tac atc ccg cac ttc cgc gag ggc 912
 Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly
 290 295 300

 gag tgg aac tgg ctg cgc gcc gcg ctt tgc acg gtg gac cgg tgc ttc 960
 Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
 305 310 315 320

 ggc ccg ttc ctc gac tgc gtg gtg cac cgc atc gtg gac acg cac gtg 1008
 Gly Pro Phe Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val
 325 330 335

 tgc cac cac atc ttc tcc aag atg ccg ttc tac cac tgc gag gag gcc 1056
 Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
 340 345 350

 acg aac gcc atc aag ccg ctg ctg gcc aag ttc tac ctc aag gac acg 1104
 Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr
 355 360 365

 acg ccc gtg ccc gtc gcg ctc tgg cgg tcc tac acg cac tgc aag ttc 1152
 Thr Pro Val Pro Val Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
 370 375 380

 gtc gag gac gac gcc aag gtc gtc ttc tac aag aac aag ctc taa 1197
 Val Glu Asp Asp Gly Lys Val Val Phe Tyr Lys Asn Lys Leu
 385 390 395

<210> 26

<211> 398

<212> PRT

<213> Phytophthora sojae

<400> 26

Met Ala Ile Leu Asn Pro Glu Ala Asp Ser Ala Ala Asn Leu Ala Thr
 1 5 10 15

Asp Ser Glu Ala Lys Gln Arg Gln Leu Ala Glu Ala Gly Tyr Thr His
 20 25 30
 Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Glu Leu Pro His Phe Ser
 35 40 45
 Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
 50 55 60
 Phe Val Thr Ser Thr Tyr Tyr Met Ile Lys Asn Val Leu Thr Cys Ala
 65 70 75 80
 Ala Leu Phe Tyr Ala Ala Thr Phe Ile Asp Arg Ala Gly Ala Ala Ala
 85 90 95
 Tyr Val Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
 100 105 110
 Gly Val Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
 115 120 125
 Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
 130 135 140
 Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
 145 150 155 160
 Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
 165 170 175
 Ser Val Leu Ala Ser Ser Trp Asn Glu Thr Leu Glu Asp Ser Pro Leu
 180 185 190
 Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro
 195 200 205
 Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
 210 215 220
 Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ala Asp Arg Glu
 225 230 235 240
 Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Ala Met Leu Ala
 245 250 255
 Val Leu Ala Ala Leu Val His Thr Phe Ser Phe Asn Thr Met Val Lys
 260 265 270

Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
 275 280 285

Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly
 290 295 300

Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
 305 310 315 320

Gly Pro Phe Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val
 325 330 335

Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
 340 345 350

Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr
 355 360 365

Thr Pro Val Pro Val Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
 370 375 380

Val Glu Asp Asp Gly Lys Val Val Phe Tyr Lys Asn Lys Leu
 385 390 395

<210> 27

<211> 24

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(24)

<223> primer

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 gccatggccc ccatcgagac cgac

24

<210> 28

<211> 20

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<400> 28

ttagcccatg tggacggaca

20

<210> 29

<211> 25

<212> DNA

<213> unknown

<220>

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<222> (1)..(25)

<223> primer

<400> 29

accatggtgg atggcccaa gacca

25

<210> 30

<211> 25

<212> DNA

<213> unknown

<220>

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<221> misc_feature

<222> (1)..(25)

<223> primer

<400> 30

ttacatggcc gggaactcga gcagg

25

<210> 31

<211> 22

<212> DNA

<213> unknown

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<222> (1)..(22)

<223> primer

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gccatggcga tcctgaaccc gg

22

<210> 32

<211> 19

<212> DNA

<213> unknown

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<222> (1)..(19)

<223> primer

<400> 32

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19

<210> 33

<211> 23

<212> DNA

<213> unknown

<220>

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<222> (1)..(23)

<223> primer

<400> 33

gccatggcgt ccaagcagga gca

23

<210> 34

<211> 25

<212> DNA

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<222> (1)..(25)

<223> primer

<400> 34

tcagttggcc ttagtcttgg tcgcc

25

<210> 35

<211> 24

<212> DNA

<213> unknown

<220>

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<222> (1)..(24)

<223> primer

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24

<210> 36

<211> 28

<212> DNA

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<220>

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<220>

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<222> (1)..(28)

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<400> 36
ttactgcgtc ttcttgccga ccgcagcg

28

<210> 37

<211> 23

<212> DNA

<213> unknown

<220>

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<400> 37

gccatggcgt cggagctgct gca

23

<210> 38

<211> 21

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(21)

<223> primer

<400> 38

ttagagggttc ttcttgccg g

21

<210> 39

<211> 22

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(22)

<223> primer

<400> 39
accatgtcgg ccgacctgct gc

22

<210> 40

<211> 18

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

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<222> (1)..(18)

<223> primer

<400> 40
ttagagcttc ttcttggc

18

<210> 41

<211> 32

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(32)

<223> primer

<400> 41
gcggccgcgc catggcccc atcgagaccg ac

32

<210> 42

<211> 28

<212> DNA

<213> unknown

<220>

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<220>

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<400> 42

gcggccgctt agcccatgtg gacggaca

28

<210> 43

<211> 33

<212> DNA

<213> unknown

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<222> (1)..(33)

<223> primer

<400> 43

gcggccgcac catggtggat ggccccaaga cca

33

<210> 44

<211> 33

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(33)

<223> primer

<400> 44

gcggccgctt acatggccgg gaactcgagc agg

33

<210> 45

<211> 30

<212> DNA

<213> unknown

<220>

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<221> misc_feature

<222> (1)..(30)

<223> primer

<400> 45

gcggccgcgc catggcgatc ctgaaccgg

30

<210> 46

<211> 27

<212> DNA

<213> unknown

<220>

<223> artificial sequence

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<221> misc_feature

<222> (1)..(27)

<223> primer

<400> 46

gcggccgcta gagcttggtc ttgtaga

27

<210> 47

<211> 31

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(31)

<223> primer

<400> 47

gcggccgcgc catggcggtc aagcaggagc a

31

<210> 48

<211> 33

<212> DNA

<213> unknown

<220>

<223> artificial sequence

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<222> (1)..(33)

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gcggccgctc agttggcctt agtcttggtc gcc

33

<210> 49

<211> 32

<212> DNA

<213> unknown

<220>

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<222> (1)..(32)

<223> primer

<400> 49

gcggccgcaa gatggagacg accttcgcgc gc

32

<210> 50

<211> 36

<212> DNA

<213> unknown

<220>

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<220>

<221> misc_feature

<222> (1)..(36)

<223> primer

<400> 50

gcggccgctt actgcgcttt cttggcgacc gcagcg

36

<210> 51

<211> 31

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(31)

<223> primer

<400> 51

gcggccgcgc catggcgtcg gagctgctgc a

31

<210> 52

<211> 29

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(29)

<223> primer

<400> 52

gcggccgctt agaggttctt cttggccgg

29

<210> 53

<211> 30

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(30)

<223> primer

<400> 53

gcggccgcac catgtcggcc gacctgctgc

30

<210> 54

<211> 26

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(26)

<223> primer

<400> 54

gcggccgctt agagcttctt cttggc

26

<210> 55

<211> 60

<212> DNA

<213> unknown

<220>

<223> artificial sequence

<220>

<221> misc_feature

<222> (1)..(60)

<223> primer

<400> 55

gtcgacccgc ggactagtgg gccctctaga cccgggggat ccggtctgc tggctatgaa 60

Patentkrav

1. Nukleinsyre kodende for et polypeptid med Δ -12-desaturaseaktivitet, idet nukleinsyren er udvalgt af gruppen
5 bestående af:
- a) en nukleinsyre med den i SEQ ID NO: 25 viste sekvens eller
 - b) nukleinsyrer, som koder for et polypeptid med den i SEQ ID NO: 26 viste sekvens eller
 - c) nukleinsyrer, som koder for et polypeptid med i det mindste
10 70 % identitet på aminosyreniveau med SEQ ID NO: 26, eller
 - d) en nukleinsyre, som er opnåelig med amplifikation af *Phytophthora sojae* med primerparret (i) SEQ ID NO. 45 og SEQ ID NO. 46 eller (ii) SEQ ID NO. 31 og SEQ ID NO. 32.
- 15 2. Protein kodet fra en nukleinsyre ifølge krav 1.
3. Genkonstrukt indeholdende en nukleinsyre ifølge krav 1, idet nukleinsyren er forbundet funktionsdygtigt med et eller flere regulationssignaler.
- 20 4. Genkonstrukt ifølge krav 3, kendetegnet ved, at nukleinsyrekonstruktet indeholder yderligere biosyntesegener fra fedtsyre- eller lipidstofskiftet udvalgt af gruppen acyl-CoA-dehydrogenase(r), acyl-ACP[= acyl carrier protein]-desaturase(r), acyl-ACP-thioesterase(r), fedtsyre-acyl-transferase(r), acyl-CoA:lysophospholipid-acyltransferase(r), fedtsyre-synthase(r), fedtsyre-hydroxylase(r), acetyl-coenzym A-carboxylase(r), acyl-coenzym A-oxidase(r), fedtsyre-desaturase(r), fedtsyre-acetylenaser, lipoxygenaser,
25 triacylglycerol-lipaser, allenoxid-syntaser, hydroperoxid-lyaser eller fedtsyre-elongase(r).
5. Genkonstrukt ifølge krav 3 eller 4, kendetegnet ved, at nukleinsyrekonstruktet indeholder yderligere biosyntesegener
35 fra fedtsyre- eller lipidstofskiftet udvalgt af gruppen af Δ -4-desaturaser, Δ -5-desaturaser, Δ -6-desaturaser, Δ -8d, Δ -12-desaturaser eller Δ -9-elongaser.

6. Vektor indeholdende en nukleinsyre ifølge krav 1 eller et genkonstrukt ifølge et af kravene 3 til 5.

7. Ikke-human organisme, transgen for i det mindste et genkonstrukt ifølge et af kravene 3 til 5 eller en vektor ifølge krav 6 eller nukleinsyren ifølge krav 1.

8. Transgen ikke-human organisme indeholdende i det mindste en nukleinsyre ifølge krav 1, et genkonstrukt ifølge et af kravene 3 til 5, en vektor ifølge krav 6 eller en organisme ifølge krav 7, idet organismen er en plante.

9. Transgen plante ifølge krav 8, kendetegnet ved, at planten er en oliefrugtplante eller nytteplante fortrinsvis udvalgt af gruppen jordnød, raps, canola, solsikke, saflor (Carthamus tinctoria), valmue, sennep, hamp, ricinus, oliven, sesam, morgenfrue, granatæble, natlys, kongelys, tidsel, hasselnød, mandel, macadamia, avocado, laurbær, græskar, hør, soja, pistacie, hjulkrone, oliepalme, kokosnød, valnød, majs, hvede, rug, havre, triticales, ris, byg, bomuld, maniok eller peber.

10. Transgen del af en transgen plante ifølge krav 8 eller 9, fortrinsvis transgene frø fra en transgen plante ifølge krav 8 eller 9.

11. Fremgangsmåde til isolering og fremstilling af olie, fedt, lipider og/eller frie fedtsyrer, idet fremgangsmåden omfatter presning eller ekstrahering af plantedelene eller plantefrøene ifølge krav 10.

12. Fremgangsmåde til fremstilling af foderstoffer, næringsmidler, kosmetika eller farmaceutika indeholdende trinnene i fremgangsmåden ifølge krav 11 og sammenblanding af disse olier, lipider og/eller frie fedtsyrer med animalske, mikrobielle eller vegetabiliske olier, lipider eller fedtsyrer.

13. Anvendelse af nukleinsyren ifølge krav 1, den transgene

plante ifølge krav 9 eller den transgene del af en transgen plante ifølge krav 10 til fremstilling af olie, fedt, lipider eller frie fedtsyrer.

- 5 14. Anvendelse af nukleinsyren ifølge krav 1, den transgene
plante ifølge krav 9 eller den transgene del af en transgen
plante ifølge krav 10 til fremstilling af kemisk ren
flerumættet fedtsyre eller fedtsyresammensætning til
anvendelser inden for levnedsmiddelindustrien,
10 kosmetikindustrien eller farmaindustrien.

